Nasal hyperreactivity in allergic rhinitis and chronic rhinosinusitis with polyps: a role for neuronal pathways

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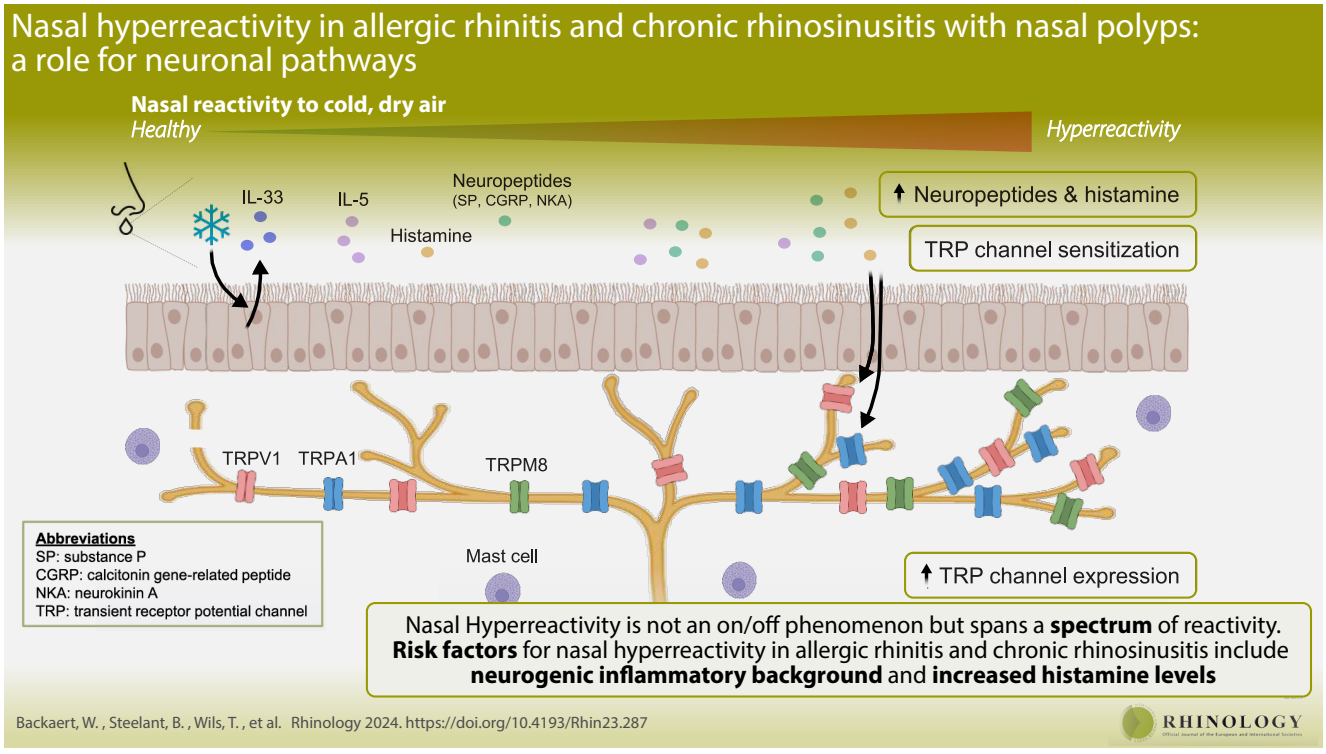
Abstract

Background: Nasal hyperreactivity (NHR) is prevalent in all chronic upper airway inflammatory phenotypes, including allergic rhinitis (AR) and chronic rhinosinusitis with nasal polyps (CRSwNP). Although NHR in patients with non-allergic rhinitis is mediated by neuronal pathways, AR and CRSwNP are mainly characterized by type 2 inflammation. **Methods**: Eighteen healthy controls and 45 patients with symptomatic AR/CRSwNP underwent a cold, dry air (CDA) provocation test for objective diagnosis of NHR. Before and after, questionnaires were filled out and nasal secretions and biopsies were collected. Markers for neurogenic inflammation (substance P, calcitonin gene-related peptide, neurokinin A), epithelial activation (IL-33), and histamine were measured in secretions by ELISA; and expression of neuronal markers PGP9.5, TRPV1, and TRPM8 was studied in biopsies by RT-q-PCR. Effects of histamine on TRPV1/A1 were studied with Ca^{2+}-imaging using murine trigeminal neurons. **Results**: CDA-provocation reduced peak nasal inspiratory flow (PNIF) of patients with subjective NHR but not of non-NHR controls/patients. Subjective (subjectively reported effect of CDA) and objective (decrease in PNIF) effects of CDA were significantly correlated. Levels of neuropeptides and histamine in nasal secretions and mRNA expression of PGP9.5, TRPV1, and TRPM8 correlated with CDA-induced PNIF-reduction. CDA-provocation induced an increase in IL-33-levels. Both TRPV1 and TRPA1 expressed on afferent neurons were sensitized by exposure to histamine. **Conclusion**: NHR is not an on/off phenomenon but spans a continuous spectrum of reactivity. A neurogenic inflammatory background and increased histamine-levels are risk factors for NHR in AR/CRSwNP.

Key words: allergic rhinitis, chronic rhinosinusitis with nasal polyps, nasal hyperreactivity, neurogenic inflammation, transient receptor potential channels

Graphical abstract



Introduction

Nasal hyperreactivity (NHR) is defined as the induction of nasal symptoms upon exposure to particular environmental stimuli such as temperature/humidity changes or air-conditioning [1]. In contrast to nasal symptoms induced as a physiological reflex, symptoms related to NHR persist longer than 10 minutes [2].

NHR is mostly studied in patients with non-allergic rhinitis (NAR) where it is found in 47.4-66.9 % of patients [1–5]. In prevalence studies, NHR is often diagnosed by means of a subjective questionnaire. Objective diagnosis, however, requires a cold (< -10°C), dry (< 10 % relative humidity) air (CDA) provocation test. A CDA-induced decrease in peak nasal inspiratory flow (PNIF) of ≥ 20 % is considered diagnostic for objective NHR [6].

Mainly neuronal pathways are suspected to contribute to the pathophysiology of NHR [1,7]. Afferent sensory neurons contain neuropeptides such as substance P (SP), calcitonin gene-related peptide (CGRP), and neurokinin A (NKA) [8]. It is currently supposed that these neuropeptides are released upon strong activation of sensory neurons. This neurogenic inflammation induces vasodilation and increased mucus secretion, ultimately resulting in nasal symptoms like nasal obstruction and rhinorrhea [1,8].

Several studies indicate an important contribution of the nociceptive transient receptor potential (TRP) channels to activation of sensory neurons [9,10]. In patients with NAR, an upregulation of the TRP Vanilloid 1 (TRPV1) – SP axis was observed [11]. These patients also harbor an increased sensitivity for the TRPV1 and TRP Ankyrin 1 (TRPA1) channel agonist allyl isothiocyanate which can be abrogated by nasal treatment with the potent and highly specific TRPV1-agonist capsaicin, along with decreased NHR [5]. Lastly, TRPV1, TRPA1, and TRP Melastatin 8 (TRPM8) were shown to contribute to bronchial hyperreactivity in patients with asthma [12–14].

Yet NHR is not limited to patients with NAR. Indeed, patients with allergic rhinitis (AR) have more nasal obstruction, rhinorrhea, itch, sneezing, pain, and plasma extravasation when challenged with capsaicin [15–18]. This is even more outspoken during the pollen season in patients with seasonal AR [19]. Other studies reported a high prevalence of NHR in AR, chronic rhinosinusitis (CRS) without (sNP) and with nasal polyps (wNP), and mixed phenotypes where more than 1 upper airway pathology is present in the same patient [2,4,20–22]. However, while NHR in NAR seems to be mediated by neuronal mechanisms, type 2 inflammation with increased levels of histamine or IL-5 is an important corner stone in the pathophysiology of AR and CRSwNP in the Caucasian population [23–26].

Some studies indicate a possible interaction between these seemingly distinct pathophysiological entities. The mast cell mediator histamine was shown to sensitize TRPV1 and TRPA1 in dorsal root ganglionic neurons [27–29]. Indeed, exposure of TRPV1$^+$ or TRPA1$^+$ neurons arising from the gut to histamine, induced an increase in the number of neurons responding to low concentrations of TRPV1-agonist capsaicin or TRPA1-agonist cinnamaldehyde[27,28]. Moreover, histamine was shown to increase TRPV1-dependent mechanosensitivity in sensory afferents arising in the bladder [29].

We therefore investigated possible mediators involved in NHR in AR and CRSwNP. We designed a prospective study to investigate the presence of neurogenic inflammation in AR and CRSwNP. A possible interaction between histamine and nociceptive trigeminal neurons was studied in vitro.

Materials and methods

Study participants

From February 2020 until December 2021, patients with symptomatic persistent AR or CRSwNP and healthy controls were recruited from the outpatient rhinology clinic of the University Hospitals Leuven. All participants were well-characterized by an otorhinolaryngologist and underwent a skin prick test and nasal endoscopy. In- and exclusion criteria are further detailed in the Supplement and Table S1.

Study design

All participants were screened for eligibility and willingness to participate by phone call. The study comprised two visits at the Department of Otorhinolaryngology at the University Hospitals Leuven at least 3 weeks apart (Figure 1A). Participants did not use any nasal medication, intranasal/oral corticosteroids, intranasal/oral antihistamines, or saline lavages during the study, starting 1 week prior to visit 1. None of the patients was on treatment with biologicals.

During visit 1, participants scored nasal symptom severity on a 100 mm-long visual analogue scale (VAS) and baseline biopsies were taken bilaterally from the inferior turbinate. On study visit 2, a CDA provocation was performed. Before and after, symptom severity was scored, PNIF was measured, and nasal secretions were harvested. Biopsies were taken bilaterally from the inferior turbinate after provocation.

All participants gave informed consent. The study was approved by the Ethical Committee Research of University Hospitals Leuven (S63139) and registered on clinicaltrials.gov (NCT04286542).

Assessment of symptom severity and nasal hyperreactivity

Patient-reported symptom scores for total nasal symptoms, nasal obstruction, rhinorrhea or post-nasal drip, nasal itch, sneezing, facial pressure, and loss of smell were indicated on 100 mm-long VAS.

Participants were considered to suffer from self-reported NHR (sNHR) in a dichotomous way (yes/no) in case of 1) increased nasal symptoms upon encounter to specific environmental triggers (temperature/humidity changes, air-conditioning, (cigarette) smoke, strong odors) reported by the patients which 2) lasts for

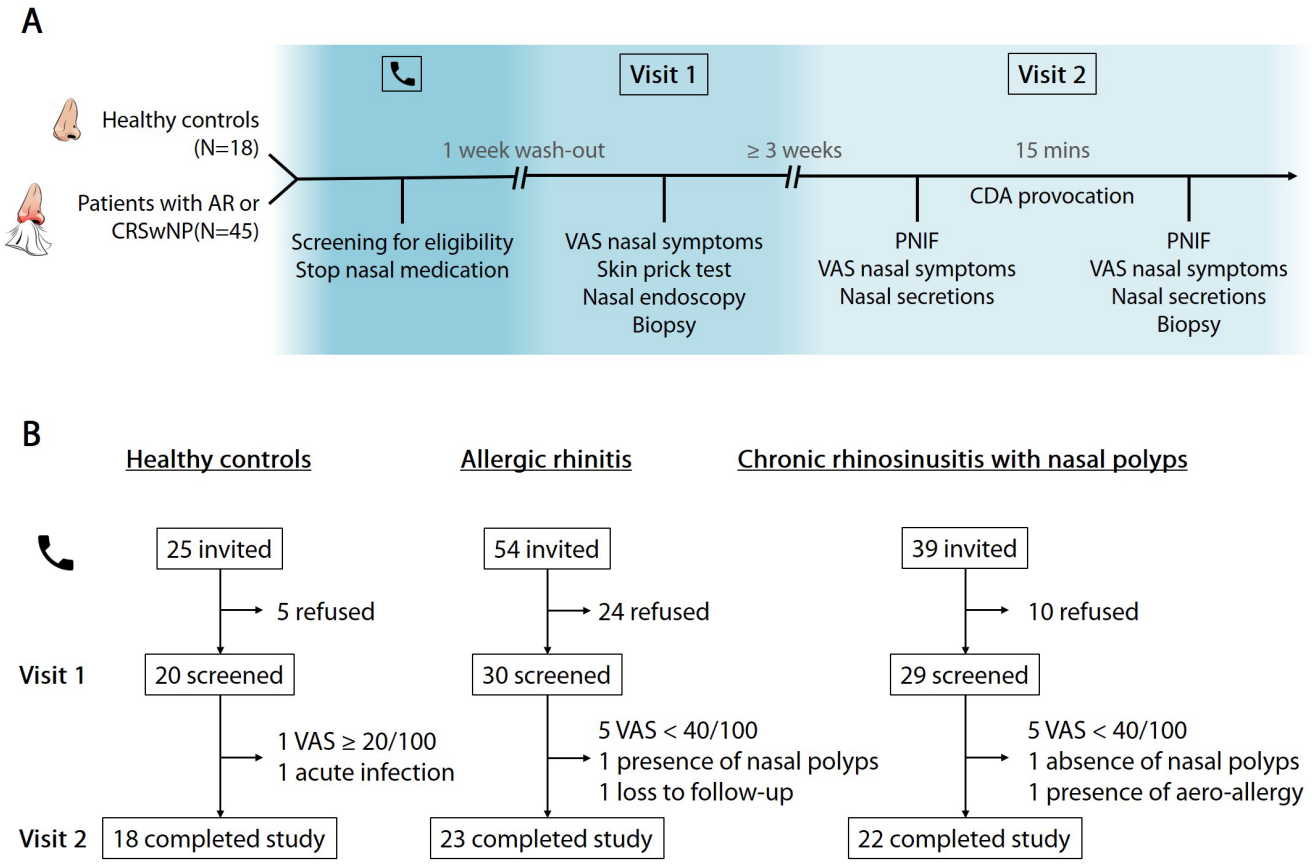


Figure 1. Study design (A) and in-/exclusion chart (B). AR: allergic rhinitis, CRSwNP: chronic rhinosinusitis with nasal polyps, PNIF: peak nasal inspiratory flow, VAS: visual analogue scale.

more than 10 minutes [2]. After a CDA provocation test, participants indicated to what degree the CDA exposure exacerbated their symptoms using VAS, allowing to study subjective NHR as a continuous parameter (Figure 2A).

For diagnosis of objective NHR (oNHR), participants underwent a CDA provocation test. oNHR was diagnosed (yes/no) in case PNIF decreased ≥ 20 % during CDA provocation [6]. The relative change in PNIF during CDA was used to objectively study reactivity to CDA in a continuous manner.

Collection of nasal secretions and biopsies of nasal mucosa

Nasal secretions were collected by insertion of nasal sponges in both nostrils for 10 minutes, allowing for absorption of secretions from the nasal cavity. Biopsies were harvested from the inferior turbinate after local anesthesia. Sampling procedures are further detailed in the supplement.

Measurement of SP, NKA, CGRP, IL-4, IL-5, IL-13, IL-33, and histamine in nasal secretions

Protein levels were measured in nasal secretions using commercially available kits. Total protein concentration was determined with a bicinchoninic acid assay (23225, Thermo Fisher Scientific, Waltham, MA, USA) according to manufacturer's instructions. The insertion of a nasal sponge could induce nasal secretions, diluting the total protein concentration (Figure S1). Therefore, protein levels in nasal secretions were corrected for total protein concentration ([concentration of protein of interest]/[total protein concentration]).

RT-q-PCR for *TRPV1, TRPA1, TRPM8, TAC1, PGP9.5, ZO-1, OCLN*, and *CLDN1* on nasal mucosal biopsies

mRNA-levels of *TRPV1, TRPA1, TRPM8, TAC1, PGP9.5, ZO-1, OCLN*, and *CLDN1* in nasal mucosa was measured by RT-q-PCR, as detailed in the supplement.

RT-q-PCR for *Trpv1*, *Trpa1*, and *Tac1* on murine trigeminal ganglia

Trigeminal ganglia were isolated from wild type C57Bl/6J mice. After overnight incubation in normal or IL-33-enriched medium, *Trpv1*-, *Trpa1*-, and *Tac1*-expression was measured by RT-q-PCR.

Calcium imaging experiments on murine trigeminal ganglionic neurons

Murine trigeminal ganglia were mechanically disrupted to obtain single neuron suspensions. After overnight incubation, Ca^{2+}

A

NHR	On/off	Continuous
Subjective	sNHR: 2 questions	VAS effect CDA
Objective	oNHR: ΔPNIF ≤ -20 %	ΔPNIF

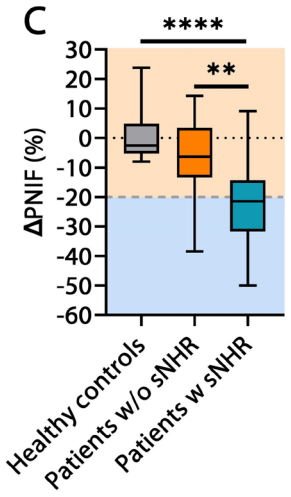
B

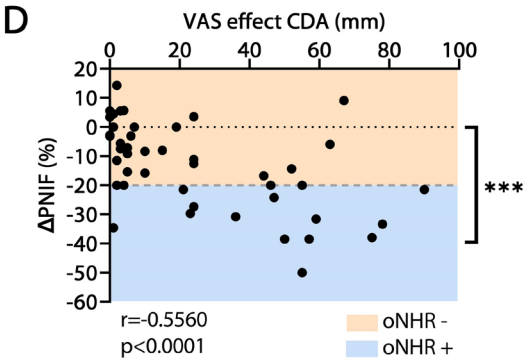
Controls

	No oNHR	oNHR
No sNHR	17	0
sNHR	1	0

Patients

	No oNHR	oNHR
No sNHR	22	4
sNHR	6	13





E

Correlation of ΔPNIF and…	r	p
VAS effect CDA	-0.5560	<0.0001
ΔVAS nasal obstruction	-0.4163	0.0022
ΔVAS rhinorrhea	-0.2843	0.0292
VAS total nasal symptoms	-0.1846	NS

Figure 2. Evaluation of subjective and objective nasal hyperreactivity (NHR). A) Schematic representation of how NHR was evaluated subjectively or objectively and as binary or continuous parameter. B) Relation between objective and subjective NHR in controls and patients. C) Change in peak nasal inspiratory flow (PNIF) in relation to self-reported NHR (sNHR). D) Correlation between continuous subjective and objective effect of cold, dry air provocation (CDA). P-values for correlations for all patients (regardless of presence of s/oNHR) is indicated at the bottom left, while p-values for possible differences between patients with/without oNHR are indicated next to the accolade. E) Correlations between objective decrease in PNIF and subjective increase in nasal obstruction/ rhinorrhea or baseline disease severity. C: Kruskal-Wallis test with post-hoc Dunn's multiple comparisons test; D: Spearman r test and Mann-Whitney test; E: Spearman r test; ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

imaging experiments were performed to study the reactivity to capsaicin (for TRPV1) and cinnamaldehyde (for TRPA1) in Krebs solution, after administration of histamine, and in presence of the histamine receptor 1 (HRH1)-inhibitor pyrilamine. In another set of experiments, reactivity to capsaicin was assessed after incubation in normal or IL-33-enriched medium. Methods are further detailed in the supplement.

Statistical methods

GraphPad Prism 9 software was used for data analysis (GraphPad Software, San Diego, CA, USA). Fisher's exact test was used to compare proportions. Normality of continuous variables was tested with Shapiro-Wilk test. Differences between two groups were tested using an (un)paired t-test or Mann-Whitney/Wilcoxon matched pairs-signed ranks test depending on normality and whether or not data were paired. Three or more groups were compared using Kruskal-Wallis test with post-hoc Dunn's multiple comparisons test. Correlations were tested with Spearman r test. Data are presented as median and interquartile range (IQR). Level for statistical significance was set at $p < 0.05$.

Results

Participants

Forty-five patients with chronic upper airway inflammation (AR or CRSwNP) and 18 healthy controls completed the study (Figure 1B, Table S3/S4). The patient group consisted of 23 patients with AR and 22 patients with CRSwNP.

Objective and subjective NHR are correlated and span a continuous spectrum

In 17/45 (37.8 %) patients a decrease in PNIF of ≥ 20 % was observed. In the control group, none of the subjects met this criterium for oNHR. sNHR was found in 5.6 % of the control group and in 42.2 % of patients. Moreover, in 13/19 patients with sNHR, oNHR was present (Figure 2B). The two-part question to diagnose sNHR had a sensitivity and specificity of 76.5 and 78.6 % respectively. Interestingly, not all patients with sNHR reached the -20 % cutoff for oNHR, while PNIF still decreased significantly more strongly compared to healthy controls ($p < 0.0001$) or to sNHR-negative patients ($p = 0.0046$) (Figure 2C/S2). There was a correlation between subjective reaction to CDA (i.e. severity

of nasal symptom induction by CDA exposure reported on VAS) and objective reaction (i.e. ΔPNIF) ($p < 0.0001$): the stronger the patient-reported effect of CDA provocation, the stronger the decrease in PNIF. oNHR-positive patients indicated a subjectively more severe reaction to CDA than oNHR-negative patients ($p = 0.0009$) (Figure 2D). The relative change in PNIF (ΔPNIF) correlated significantly with the subjective increase in nasal obstruction ($p = 0.0022$) or rhinorrhea ($p = 0.0292$), but not with baseline VAS total nasal symptoms ($p = 0.3214$) (Figure 2E).
These results suggest that nasal reactivity to environmental stimuli and its underlying mechanisms are part of a continuous spectrum rather than it being a binary all/none-phenomenon.

Upregulated neuronal pathways and increased histamine levels are risk factors for enhanced reactivity to cold, dry air provocation.

Having found that patients with sNHR reacted more strongly to CDA provocation, we next evaluated which mediators were involved. We measured different neuropeptides in nasal secretions of patients. A correlation between levels of the neuropeptides SP, NKA, and CGRP at baseline and objective effects of CDA provocation was observed (Figure 3A-C): the more neuropeptides measured in nasal secretions at baseline, the more severe the objective reactivity to CDA provocation was. In addition, levels of these neuropeptides strongly correlated with each other (Figure S3). Moreover, a negative correlation was observed between the ΔPNIF and mRNA expression of *TRPV1*, *TRPM8*, and *PGP9.5* in nasal biopsies (Figure 3G). Expression levels of *TRPA1* and *TAC1* were below detection limit.
Secondly, we studied if NHR was associated with epithelial cell activity. No correlation between baseline protein levels of alarmin IL-33 in nasal secretions and reactivity to CDA was observed (Figure 3D). Expression of *ZO-1* on nasal biopsies and reactivity to CDA were significantly correlated, which was not observed for other tight junction genes *OCLN* and *CLDN1* (Figure 3G).
Since type 2 inflammation is a corner stone in the pathophysiology of AR and CRSwNP, we then focused on Th2-cytokines and their possible contribution to NHR. We found a trend towards a positive correlation between IL-5 levels in nasal secretions and ΔPNIF ($p = 0.0510$) (Figure 3E). Levels of NKA and IL-5 correlated negatively, but no correlation was observed between levels of SP/CGRP and IL-5 in nasal secretions (Figure S4). Levels of IL-4 and IL-13 were below detection limit. Lastly, a significant correlation was observed between histamine levels in nasal secretions and reactivity to CDA (Figure 3F).
Collectively, these results suggest that neuronal pathways underlie reactivity to CDA provocation in AR and CRSwNP and that histamine could possibly play a modulating role in it.

Histamine sensitizes murine trigeminal ganglionic neurons to capsaicin and cinnamaldehyde

Given the observation that levels of histamine in nasal secretions correlated with reactivity to CDA, we hypothesized that histamine might lower the activation threshold of trigeminal sensory neurons. To investigate this, murine trigeminal ganglionic neurons were exposed to the TRPV1-agonist capsaicin or the TRPA1-agonist cinnamaldehyde twice, while intracellular Ca2+ concentration was monitored. When neurons were exposed to histamine in between the two applications, significantly more neurons responded to the second application of capsaicin (15.9 versus 27.0 % of $TRPV1^+$ neurons, $p = 0.0058$) or cinnamaldehyde (8.5 versus 16.9 % of $TRPA1^+$ neurons, $p = 0.0486$) (Figure 4B/D). This increase was not observed in the absence of histamine (19.2 versus 11.0 %, $p = 0.0226$ for capsaicin; 6.2 versus 8.7 %, $p = 0.5252$ for cinnamaldehyde) nor in presence of the concomitant exposure to HRH1-inhibitor pyrilamine (7.5 versus 3.8 %, $p = 0.0717$ for capsaicin; 13.1 versus 14.0 %, $p = 0.8878$ for cinnamaldehyde). These results indicate that histamine can sensitize murine trigeminal ganglionic neurons for capsaicin (TRPV1) and cinnamaldehyde (TRPA1) in a HRH1-dependent pathway.

Cold, dry air induces an increase in IL-33 in nasal secretions, but levels of neuropeptides, IL-5, and histamine remain stable

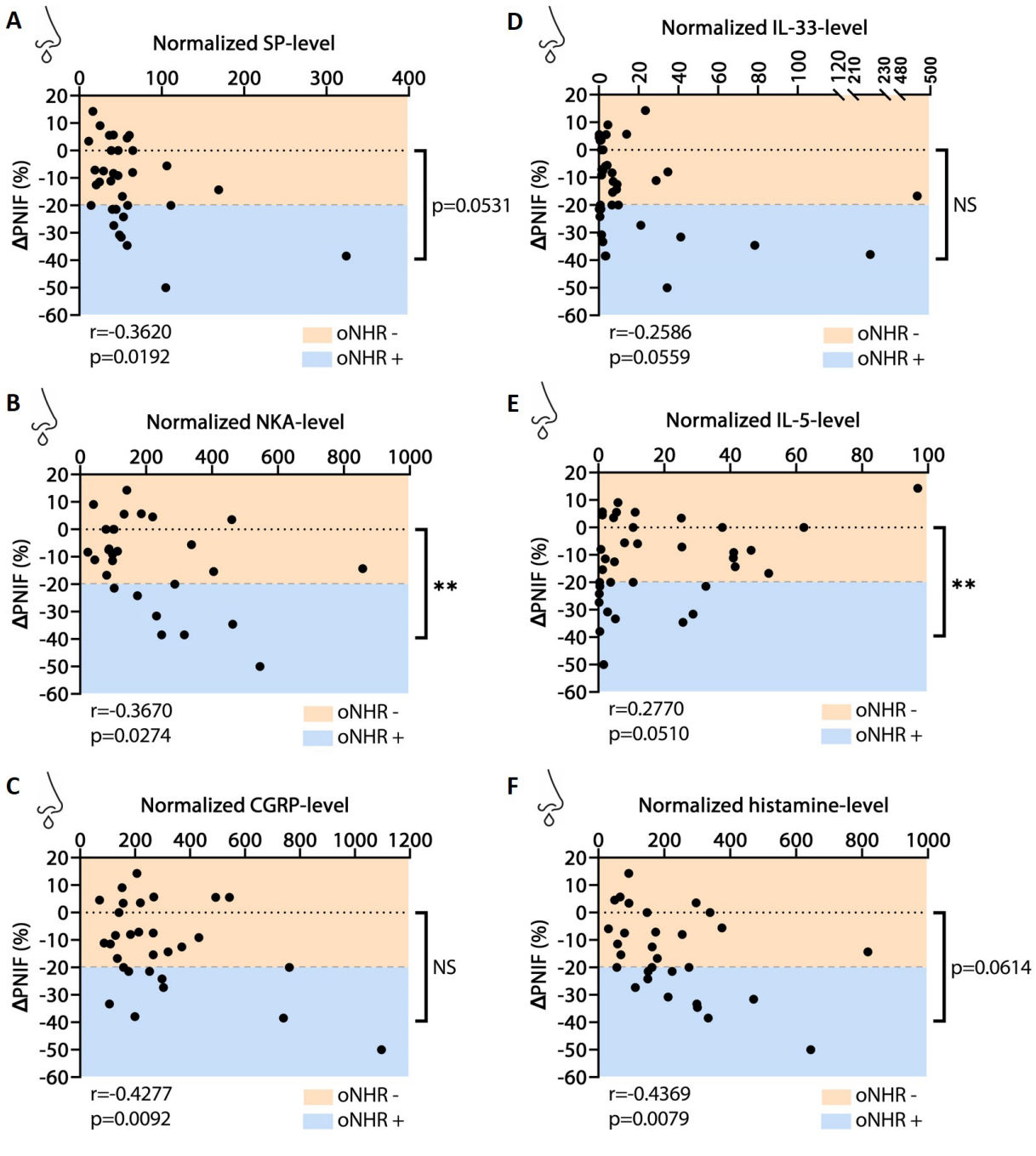
It is currently proposed that neuropeptides induce nasal symptoms in NHR, yet this has never been directly studied [1]. Therefore, we compared protein levels in nasal secretions of patients before and after CDA provocation. No difference in levels of SP, NKA, CGRP, IL-5, or histamine in nasal secretions was observed before versus after CDA provocation (Figure S5A). CDA provocation induced a significant increase in IL-33 levels in nasal secretions (median 3.63 with IQR 1.35-13.95 versus median 8.63 with IQR 2.71-47.64, $p = 0.0005$) (Figure 5). No differences in expression levels of *TRPV1*, *TRPM8*, *PGP 9.5*, *ZO-1*, *OCLN*, or *CLDN1* mRNA in nasal biopsies were observed when comparing visit 1 (baseline) with visit 2 (after CDA provocation) (Figure S5B).

IL-33 does not affect murine trigeminal sensitivity to capcaicin

Since IL-33 levels are increased in nasal secretions after CDA provocation, we investigated whether it affects neuronal sensitivity. After exposure of murine trigeminal ganglia to IL-33 during overnight incubation, similar levels of expression of *Tac1*, *Trpv1*, and *Trpa1* were observed when compared with the medium condition. Similarly, the percentage of neurons responding to capsaicin 10 nM was equal in both groups (Figure 6).

Discussion

The mechanisms underlying NHR have been almost exclusively studied in patients with NAR [1]. Recently, explorative studies



G

Correlation of ΔPNIF and …	*TRPV1*	*TRPM8*	*PGP9.5*	*ZO-1*	*OCLN*	*CLDN1*
r	-0.3390	-0.3263	-0.3188	0.3007	-0.1878	-0.1176
p	0.0187	0.0228	0.0255	0.0353	NS	NS

Figure 3. Endotypic background and reactivity to cold, dry air. A-F) Correlations between baseline protein levels in nasal secretions and objective reactivity to cold, dry air measured by change in peak nasal inspiratory flow (ΔPNIF). P-values for the correlations are found at the bottom-left of each graph, while p-values for possible differences between oNHR+ and oNHR- patients are indicated next to the accolade. G) Correlation between change in PNIF and baseline expression levels in nasal mucosal biopsies. (A-G: Spearman r test; A-F: Mann-Whitney test; ** $p < 0.01$). sNHR: subjective nasal hyperreactivity, oNHR: objective nasal hyperreactivity, SP: substance P, NKA: neurokinin A, CGRP: calcitonin gene-related peptide, IL: interleukin, TRPV1: transient receptor potential channel vanilloid 1, TRPM8: transient receptor potential channel melastatin 8, PGP9.5: protein gene product 9.5, ZO-1: zonula occludens 1, OCLN: occludin, CLDN1: claudin 1.

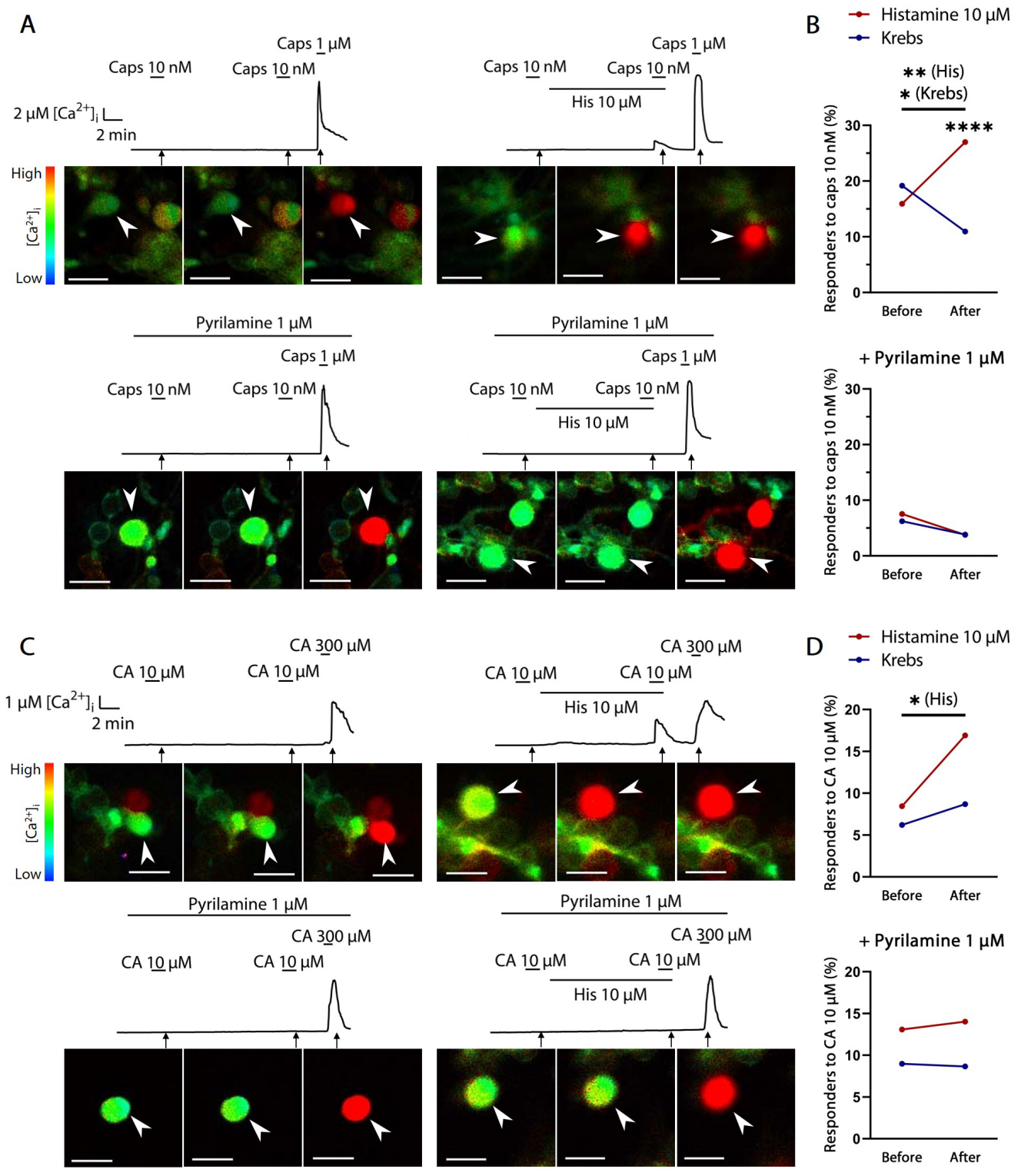


Figure 4. Calcium imaging experiments in murine trigeminal ganglionic neurons. A and C) Representative time courses of intracellular Ca^{2+} changes in single trigeminal neurons (white arrowheads) (scale bar = 20 µm). B) Percentage of neurons responding to application of capsaicin (caps) 10 nM before and after exposure to Krebs or histamine (N = 219 neurons (control), N = 226 (histamine 10 µM), N = 209 (control + pyrilamine 1 µM), and N = 292 (histamine 10 µM + pyrilamine 1 µM)). D) Percentage of neurons responding to application of cinnamaldehyde (CA) 10 µM (N = 161 neurons (control), N = 142 (histamine 10 µM), N = 312 (control + pyrilamine 1 µM), and N = 214 (histamine 10 µM + pyrilamine 1 µM)). (B and D: Fisher's exact test, * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$.) His: histamine.

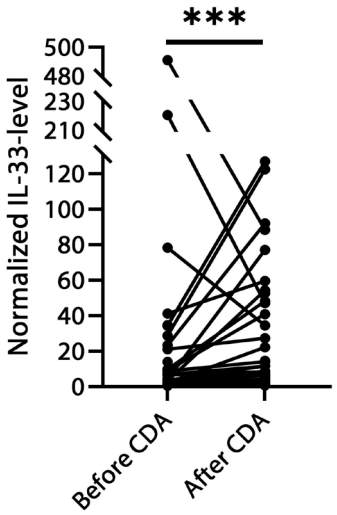


Figure 5. Effect of cold, dry air provocation (CDA) on interleukin (IL) 33 levels. (Wilcoxon matched-pairs signed rank test, *** $p < 0.001$.)

showed that NHR is not limited to NAR, but can also be observed in other phenotypes of chronic upper airway inflammation, such as AR and CRSwNP which are both mainly characterized by type 2 inflammatory pathways [2,4,21]. We therefore investigated the pathophysiology of NHR in AR and CRSwNP. We report a prevalence of sNHR of 5.6 % in healthy controls and 42.2 % in patients with AR or CRSwNP (Figure 2B). This is in line with previous reports using a similar definition of NHR and highlights the not to be underestimated prevalence of NHR [2].
NHR is often seen as an on/off phenomenon, which is either assessed subjectively by means of two questions, or objectively by a CDA provocation test [2,6]. From a mechanistic point of view, this dichotomous approach is unlikely to sufficiently reflect intercellular interactions and continuous biological responses.

Moreover, the reported prevalence of sNHR differed over various studies [2–5,21]. Many of these are questionnaire-based studies, providing room for variation due to use of different definitions. Even though not all sNHR-positive patients reached the threshold of a decrease in PNIF of ≥ 20 % in the current study, their reaction to CDA provocation is stronger compared to patients without sNHR (Figure 2B/C). This observation illustrates the continuous nature of NHR and its underlying mechanisms rather than it being an on/off phenomenon.
Segboer et al. reported a decrease in PNIF after CDA provocation in patients with AR/NAR, while also describing a high prevalence of sNHR in these patient groups [4]. Our study now confirms a correlation between sNHR and oNHR (Figure 2C). Notably, oNHR was observed in only 53.8 % of patients with AR and sNHR, compared with 100 % of patients with CRSwNP and sNHR (Figure S6). This subjective overestimation in the AR-group could be due to the intermittent nature of the disease where symptoms of NHR might be confused with symptoms of an acute allergic reaction.
Similarly, we observed a correlation between subjective and objective continuous measurements of reactivity to CDA (Figure 2D-E). Indeed, changes in PNIF correlated with the subjective effect of CDA and subjective changes in nasal obstruction, illustrating the validity of the CDA provocation test. Notably, PNIF only reflects nasal obstruction, while we here confirm a previous observation that environmental triggers can induce subjectively increased rhinorrhea or postnasal drip [2]. Increased nasal secretions may contribute to nasal obstruction, but objective measurements of nasal secretions could further improve the diagnostic CDA provocation test. Lastly, it was previously reported that intranasal capsaicin provocation provoked more severe symptoms during compared to outside of the pollen season in patients with seasonal AR [18,19]. However, these studies

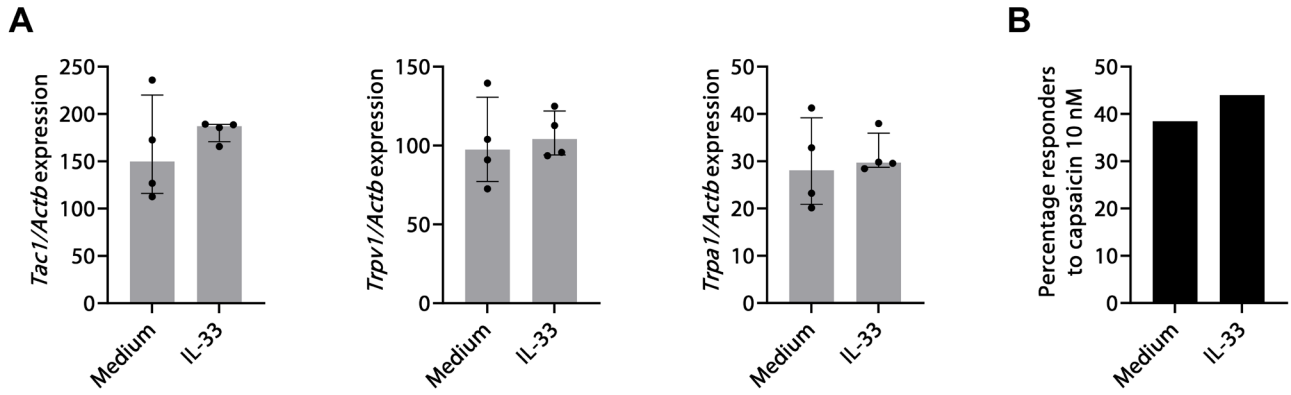


Figure 6. Effect of interleukin (IL) 33 on murine trigeminal function. A) Expression of neurogenic markers Tac1 (Tachykinin precursor 1), Trpv1 (Transient receptor potential channel vanilloid 1), and Trpa1 (Transient receptor potential channel ankyrin 1) relative to Actb (β-actin). B) Percentage of neurons responding to application of capsaicin 10 nM (N = 278 (medium), N = 334 (IL-33)). (Mann-Whitney test, not significant.)

were rather small, covering only 11 and 13 patients, and we did not observe a correlation between subjective baseline disease severity and reactivity to the clinically more relevant and more sensitive/specific CDA provocation [1]. Hence, NHR seems to affect patients with various levels of disease control.

While NHR is suspected to result from neurogenic inflammation in NAR, AR and CRSwNP are classically featured by type 2 inflammation [1,24,30,31]. Our data show a correlation between reactivity to CDA and levels of neuropeptides SP, NKA, and CGRP, expression levels of the nociceptors TRPV1 and TRPA1, and expression levels of neuronal marker PGP9.5 (Figure 3A-C/G). This means that the higher the levels of neuronal markers were, the stronger the reaction to CDA provocation was. Thus, this study shows that also neuronal pathways are possible in AR or CRSwNP and that they correlate with NHR.

Allergens and antigens can more easily penetrate to the submucosa in AR and CRSwNP due to protease- or immune-induced barrier defects [32,33]. Therefore, it is plausible that epithelial barrier defects might facilitate the exposure of nerve endings to external stimuli, hence contributing to NHR. We could not observe a clear relationship between expression levels of tight junction proteins and reactivity to CDA (Figure 3G). NHR in AR and CRSwNP seems to be unrelated to barrier (dys)function, as it is in NAR [34].

NHR is suspected to be mediated by neuronal pathways, but a potential interaction with type 2 inflammation is unknown. Therefore, we also measured type 2 signature cytokines IL-4, IL-5, and IL-13 in nasal secretions. Patients with oNHR exhibited significantly lower IL-5 levels compared with those without (Figure 3E). Since no differences in subjective disease severity could be observed, we hypothesized that type 2 and neurogenic inflammation are inversely correlated. However, only NKA levels correlated negatively with IL-5 levels, and not SP or CGRP (Figure S4). Given the lack of an unambiguous correlation between neuropeptides and IL-5, the effects of IL-5 – or by extension type 2 inflammation – on reactivity to CDA remain unclear at this point.

We observed a positive correlation between levels of the mast cell mediator histamine in nasal secretions and objective reactivity to CDA provocation (Figure 3F). Moreover, we showed that histamine sensitized murine trigeminal ganglionic neurons for capsaicin and cinnamaldehyde (Figure 4). The mast cell mediator histamine was shown to sensitize dorsal root ganglionic neurons for capsaicin and cinnamaldehyde in the context of irritable bowel syndrome or detrusor overactivity [27–29,35]. Considering NHR in AR or CRSwNP, one cannot only speak of upregulation of nociceptor expression, but also of neurogenic sensitization, at least in murine neurons. This could occur by direct sensitization and/or by recruitment of TRP channels to the plasma membrane [36,37].

Several mediators, such as CGRP or histamine, can exert vasodilatory effects, which consequently lead to nasal obstruction [9,36,38]. Their levels in nasal secretions, however, remained constant over CDA provocation. Interestingly, CDA provocation only induced an increase in IL-33 levels in nasal secretions, reflecting potential epithelial activation (Figure 5/S5).

IL-33 is known to elicit itch in atopic dermatitis, probably via sensitization of sensory neurons [39]. Also, TRPV1$^+$ neurons are implicated in itch pathways [40,41]. However, we failed to find evidence of a direct interaction between IL-33 and TRPV1. Admittedly, IL-33 could influence nasal reactivity to environmental triggers via other mechanisms. It has been described to induce angiogenesis and increase vascular permeability, which could contribute to nasal congestion [42]. Moreover, as an alarmin, IL-33 is known to interact with many immune cells, such as mast cells or type 2 innate lymphoid cells, which can consequently release their mediators ultimately leading to nasal symptoms [43,44]. Lastly, as an upstream regulator of IL-13 and IL-31, IL-33 could indirectly increase transcription of TRPV1/A1 in vivo, potentiating neurogenic inflammation [45].

Protein concentrations in nasal secretions are inadvertently affected by the method used to collect the nasal secretions (Figure S1). Since we observed a correlation between total protein content and volume of nasal secretions harvested or measured levels of SP, we opted to correct the measured protein levels for total protein content. Admittedly, it remains open for debate which correction method is most appropriate for various ways of collecting nasal secretions.

Even though all participants were well-characterized by otorhinolaryngologists, a limitation of our study is the small sample size, which restricts the possibility of subgroup analyses on patients with AR or CRSwNP due to a lack of power. Sampling of nasal secretions using sponges placed in the nasal cavity is a widely used technique, but has the limitation that proteins bound to receptors on the mucosal surface are probably not harvested in this way [46]. Moreover, one should keep in mind that interactions may take place not in the nasal cavity itself, where secretions are collected, but at the submucosal level. Lastly, taking mucosal biopsies comes with a mucosal healing reaction. To limit this effect, we provided a healing period of 3 weeks between visit 1 and 2 and no residual mucosal abnormalities could be observed during nasal endoscopy on visit 2. Nevertheless, possible residual microscopic alterations could not be excluded.

Conclusion

We here provide evidence that NHR manifests as a continuum across patients and that it is orchestrated by neuronal pathways and modulated by histamine. However, further research is required to investigate which specific mediators cause nasal symptoms such as nasal obstruction or increased secretions in response to environmental triggers. Future studies should therefore focus on the pathways coupling environmental exposure

with induced nasal symptoms and treatment strategies targeting the underlying neurogenic inflammation.

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Conflicts of interest

The authors declare no conflicts of interest.

Authors' contributions

WB: study design and conception, sample collection, sample processing, data interpretation, drafting the article, and revising the article for important intellectual content. BS, KT, PH, LVG: study design and conception, data interpretation, drafting the article, and revising the article for important intellectual content. ZQ, TW, ED, ACJ, BB: sample processing and revising the article for important intellectual content. MJ, RS, DB: Data interpretation and revising the article for important intellectual content.

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SUPPLEMENTARY MATERIAL

Supplementary methods

Study participants

All patients had a Visual Analogue Scale (VAS) score of ≥ 40/100 mm for any nasal symptom for at least 12 weeks (Table S1). Patients with persistent allergic rhinitis (AR) were included in the months October-December to minimize effects of potential seasonal allergies. House dust mite allergy was confirmed on study visit 1 by skin prick testing and presence of relevant nasal symptoms. Patients with chronic rhinosinusitis with nasal polyps (CRSwNP) were included in case of bilateral presence of nasal polyps seen on nasal endoscopy during visit 1. Patients with other upper airway pathologies apart from AR or CRSwNP were excluded. Additional clinical details on the participants can be found in Table S3/S4.

Healthy control subjects suffered no sinonasal symptoms, had no evidence of atopy to aero-allergens on skin prick test, and had no signs of rhinosinusitis on nasal endoscopy.

All participants were 18-65 years old, never used allergen immunotherapy, used no nasal medication for at least 1 week before the first study visit, had no relevant anatomic abnormalities in the nose contributing to nasal symptoms, and were free of acute upper airway infection.

Subjective and objective measurements of hyperreactivity

Subjective, self-reported nasal hyperreactivity (sNHR) was diagnosed in case of a positive answer to both of the questions "Are your nasal complaints triggered or exacerbated by any of the following triggers: (…)?" and "In this case, do they last longer than 10 minutes?".

For objective assessment of NHR, cold (< -10°C), dry (< 10 % relative humidity) air was delivered for 15 minutes by a custom-made cold, dry air (CDA)-device at a flow of 25 L/min via an anesthetic mask. Holes in the mask assured escape of redundant air, preventing overpressure. Stability of temperature and humidity was continuously checked with a custom-made device calibrated using a 176H datalogger (0572 1765, Testo, Ternat, Belgium). Participants inhaled strictly via the nose. Peak Nasal Inspiratory Flow (PNIF) was measured immediately before and after provocation with a PNIF-device (In-Check Nasal Inspiratory Flow Meter, Clement Clarke International, Harlow, UK). At each time point, the median of three measurements ≤ 10 % apart was used in accordance with previous studies [1,2]. In the rare instances where PNIF measurements were > 10% apart, this was in our study always due to a clear technical issue such as air leakage due to a non-airtight seal of the anesthetic mask which is pressed too softly against the face of the participant. In these cases, the clearly outlying measurements with a clear identifiable technical cause were repeated.

Collection of nasal secretions and biopsies of nasal mucosa

To collect nasal secretions, nasal sponges (Post-Op Sinus Pack K9, Q770532, Ivalon Surgical Products, Fabco, New London, CT, USA) were weighed and inserted in both nostrils for 10 minutes. Afterwards, the sponges were weighed again, and saline was added to reach a 1:3 dilution. The sponges were squeezed with a syringe and centrifuged at 500 g at 4 °C for 15 minutes. Nasal secretions were stored at -80 °C until further analysis.

At the end of visit 1 and 2, cotton balls soaked in cocaine 1 % were placed next to the inferior turbinates and left in place for 15 minutes to achieve local anesthesia. Next, a biopsy was taken from the inferior turbinate bilaterally with a Fokkens forceps. Biopsies were snap frozen in liquid nitrogen and stored at -80 °C until further analysis.

Measurement of SP, NKA, CGRP, IL-4, IL-5, IL-13, IL-33, and histamine in nasal secretions

Using commercially available ELISA-kits, levels of neuropeptides – SP (583751, Cayman Chemical, Ann Arbor, MI, USA), NKA (abx152497, Abbexa, Cambridge, UK), and CGRP (RD-CGRP-Hu, Reddot Biotech, Kelowa, BC, Canada) – and histamine (LS-F39267, Lifespan Biosciences, Seattle, WA, USA) were measured according to manufacturer's instructions. Levels of type 2 inflammatory markers IL-4, IL-5, and IL-13, and epithelial marker IL-33 were determined using the U-plex platform of Mesoscale Discovery (Mesoscale Diagnostics, Rockville, MD, USA).

RT-q-PCR for *TRPV1, TRPA1, TRPM8, TAC1, PGP9.5, ZO-1, OCLN*, and *CLDN1* on nasal mucosal biopsies

Biopsies were homogenized in RLT lysis buffer (79216, Qiagen, Hilden, Germany) using Lysing Matrix D and a FastPrep-24-device (116913100 and 116004500, MP Biomedicals, Brussels, Belgium) and RNA was extracted using the RNeasy Mini Kit (74106, Qiagen, Hilden, Germany). cDNA was obtained using a High-Capacity cDNA Reverse Transcription kit (4368814, Thermo Fisher Scientific, Waltham, MA, USA) starting from 500 ng RNA. Real-time quantitative PCR was performed for *TRPV1*, *TRPA1*, *TRPM8*, protein gene product 9.5 (*PGP9.5*), tachykinin precursor 1 (*TAC1*), zonula occludens 1 (*ZO-1*), occludin (*OCLN*), and claudin 1 (*CLDN1*) (Table S2) with the CFX Connect Real-Time PCR Detection System (Bio-rad, Hercules, CA, USA). Expression levels were normalized to the geometric mean of reference genes β-actin (*ACTB*) and guanine nucleotide-binding protein subunit beta-2-like 1 (*GNB2L1*). cDNA plasmid standards of each specific target gene were used to quantify the amount of target genes in unknown samples [3].

Isolation and culture of murine trigeminal ganglia and ganglionic neurons

Male C57BL/6 mice (8 weeks old) were obtained from Harlan (Horst, The Netherlands) and kept under conventional conditions. Experimental procedures were approved by the Ethical Committee for Animal Research at the KU Leuven (P150/2017). Trigeminal ganglia from wild type mice were bilaterally dissected and digested with collagenase and dispase (1 mg/mL and 2.5 mg/mL respectively, Thermo Fisher Scientific, Waltham, MA, USA) in Neurobasal A medium (10888022, Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10 % fetal calf serum for 40 minutes at 37°C, 5 % CO_2, similar to what was previously described for dorsal root ganglionic neurons [4]. To obtain single neuron suspensions needed for Ca^{2+} imaging experiments, the ganglia were mechanically dissociated using hollow needles with decreasing diameters. Cells were seeded on poly-ornitin (500 µg/mL) and laminin (100 µg/mL) coated glass bottom dishes (FD35-100, Fluorodish WPI, Hertfordshire, UK) and cultured for 18-24 hours at 37°C, 5 % CO_2, in Neurobasal A medium supplemented with B-27 (2 %), GlutaMAX (1 %), Penstrep (3 %), neurotrophin 4 (10 ng/mL), and glial cell line-derived neurotrophic factor (2 ng/mL) (all products bought from Thermo Fisher Scientific, Waltham, MA, USA).

RT-q-PCR for *Trpv1*, *Trpa1* and *Tac1* on murine ganglia

RNA was extracted from whole trigeminal ganglia of male C57BL/6 mice and cDNA was obtained as described higher for the human nasal mucosal biopsies. RT-q-PCR was performed for *Trpv1*, *Trpa1*, and *Tac1* and Actb was used as refence gene (Table S2).

Intracellular calcium imaging experiments

Cultured murine trigeminal ganglionic neurons (mTGNs) were loaded with 2 µM of Fura-2AM ester for 30 minutes at 37°C prior to the recordings. Alternating illumination at 340 and 380 nm by a Lambda XL illuminator (Sutter Instruments, Mont-Saint-Guibert, Belgium) evoked fluorescent signals (emission at 510 nm) that were recorded using an Orca Flash 4.0 camera (Hamamatsu Photonics Belgium, Mont-Saint-Guibert, Belgium) on a Nikon Eclipse TI fluorescence microscope (Nikon Europe, Amsterdam, The Netherlands). The ratio of the fluorescent signals to both excitation wavelengths after correction for background fluorescence was monitored using Nikon Imaging Software Elements platform (Nikon Europe, Amsterdam, The Netherlands). The intracellular Ca^{2+} concentration was calculated as described previously using a custom-written macro in Igor Pro software (WaveMetrics, Lake Oswego, OR, USA)[5].
Dishes were mounted on the microscope using a custom-made recording chamber. During the experiments, neurons were continuously perfused using a gravitation-based perfusing system, continuously refreshing the extracellular environment [6]. Experiments were performed using standard Krebs solution (150 mM NaCl, 6 mM KCl, 10 mM HEPES, 1.5 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM glucose, pH adjusted to 7.4 with NaOH) at 37°C.
After a 5-minute acclimatization period, cells were exposed twice to either the TRPV1-agonist capsaicin 10 nM or the TRPA1-agonist cinnamaldehyde 10 µM (1091108 and 239968, Sigma-Aldrich, Saint Louis, MI, USA). Between stimuli, Krebs (control) or histamine 10 µM (H0600000, Sigma-Aldrich, Saint Louis, MI, USA) was administered for 10 minutes. $TRPV1^+$ or $TRPA1^+$ neurons were identified by administration of capsaicin 1 µM or cinnamaldehyde 300 µM respectively at the end of the protocol. Similar experiments were performed in presence of Histamine receptor 1-inhibitor pyrilamine 1 µM (P5514-5G, Sigma-Aldrich, Saint Louis, MI, USA).

In another set of experiments, mTGNs were incubated overnight in normal or IL-33-enriched medium (20 ng/mL, 200-33, Peprotech, London, UK). The following day, the neurons were exposed to capsaicin 10 nM while monitoring the intracellular calcium concentration. Trigeminal ganglionic neurons of 3-4 mice were used per condition.

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Table S1. In- and exclusion criteria.

	Healthy control	Allergic rhinitis	Chronic rhinosinusitis with nasal polyps
Inclusion	• 18-65 years old • VAS score < 20/100 for all nasal symptoms*	• 18-65 years old • House dust mite-allergy based on patient history and skin prick test • VAS score of ≥ 40/100 for any nasal symptom*	• 18-65 years old • Chronic rhinosinusitis with nasal polyps based on nasal endoscopy • VAS score of ≥ 40/100 for any nasal symptom*
Exclusion	• Aero-allergy and/or (history of) use of AIT • Chronic rhinosinusitis without/with nasal polyps	• Chronic rhinosinusitis without/with nasal polyps • (History of) use of AIT	• Aero-allergy and/or (history of) use of AIT
	• Relevant anatomical abnormalities in the nose • Acute upper airway infection in the past 2 weeks • Recent (7 days) use of nasal medication intranasal/oral corticosteroids, intranasal/oral antihistamines, or saline lavages. • Alcohol consumption in the past 24 hours • Use of tricyclic antidepressants • Intranasal drug-abuse in the past 12 months • Currently participating in other clinical studies • Pregnancy or breastfeeding • Active malignancy		

*Individual VAS scores for total nasal symptoms, nasal obstruction, rhinorrhea/post-nasal drip, sneezing, nasal itch, headache/facial pain, and loss of smell. VAS: visual analogue scale (mm), AIT: allergen immunotherapy.

Table S2. Primer (FW: forward, RV: reverse) and probe (TP: taqman probe) sequences used for real-time quantitative PCR on human nasal mucosal biopsies (capitals) or murine trigeminal ganglia (lower cases).

Gene	primer	sequence
ACTB	FW	gga cat ccg caa aga cct gt
	RV	ctc agg agg agc aat gat ctt gat
	TP	ctg gcg gca cca cca tgt acc ct
GNB2L1	FW	cac tgt cca gga tga gag cca
	RV	cat acc ttg acc agc ttg tcc c
	TP	tcc gct tct cgc cca aca gca g
TRPV1	FW	aag cca tgc tca acc tgc ac
	RV	tgt ctg gcc ctt gta gta gct g
	TP	cgg aca gcc tga agg agc ttg tca a
TRPA1	FW	tcc tgc cga gac tat tat atc gag tat
	RV	gct cta tgc ggt tat ttt gta cca t
	TP	tat gaa ccg ctt aca gcc ctc aac gc
TRPM8	FW	gcc tac gtg ctg ctc atg g
	RV	cat tta cgt acc act gtc tca ctt ca
	TP	ttt cca ttc ggt gcc aca ccc c
PGP9.5	FW	agg cca atg tcg ggt aga tg
	RV	gtt cac cgg aaa agg cat tc
	TP	tgg atg gcc acc tct atg aac ttg atg g
TAC1	FW	gga ctg tcc gtc gca aaa tc
	RV	tcc tat ttc ttc tgc aaa cag ctg
	TP	aac atg aaa atc ctc gtg gcc ttg gc

Gene	primer	sequence
ZO-1	FW	gtg cct aaa gct att cct gtg agt c
	RV	cta tgg aac tca gca cgc cc
	TP	tgg cca cag ccc gag gca tat t
OCLN	FW	cca atg tcg agg agt ggg tta a
	RV	ttg cca ttg gaa gag tat gcc
	TP	ctg cag gca cac agg acg tgc c
CLDN1	FW	cca gtc aat gcc agg tac gaa t
	RV	ata ggg cct tgg tgt tgg gt
	TP	tca ggc tct ctt cac tgg ctg ggc
Actb	FW	aga ggg aaa tcg tgc gtg ac
	RV	caa tag tga tga cct ggc cgt
	TP	cac tgc cgc atc ctc ttc ctc cc
Tac1	FW	gac cgc aaa atc gaa cat ga
	RV	ttg gca tcg att tcc tct gc
	TP	atc ctc gtg gcc gtg gcg gt
Trpv1	FW	cat gat tga gaa gat gat cct cag a
	RV	ttc cca tcc tcg atc agt gtc
	TP	tgt tct tgt ttg gat ttt cca cag ccg t
Trpa1	FW	tgt gaa tgc agt tga tgg caa
	RV	tgt ctg ctc cca ctg ata tta ggt at
	TP	acc ctg ctt cac aga gcc tcg tta ttt ga

ACTB/Actb: β-actin, GNB2L1: guanine nucleotide-binding protein subunit beta-2-like 1, TRPV1/Trpv1: transient receptor potential channel vanilloid 1, TRPA1/Trpa1: transient receptor potential channel ankyrin 1, TRPM8: transient receptor potential channel melastatin 8, PGP9.5: protein gene-product 9.5, TAC1/Tac1: tachykinin precursor 1, ZO-1: zonula occludens 1, OCLN: occludin, CLDN1: claudin 1, Gapdh: glyceraldehyde-3-phosphate dehydrogenase.

Table S3. Demographic data.

	Controls (N = 18)	Patients (N = 45)	p-value
Median age (years (IQR))	42 (27-53)	41 (30-57)	0.5039[#]
Male/female	9/9	29/16	0.3938[§]
Current smokers (%)	5.6	17.8	0.4258[§]
NHR			
sNHR (%)	1 (5.6)	19 (42.2)	0.0059[§]
oNHR (%)	0 (0)	17 (37.8)	0.0014[§]

[#] Mann-Whitney test, [§] Fisher's exact test. IQR: interquartile range, sNHR: subjective nasal hyperreactivity, oNHR: objective nasal hyperreactivity.

Table S4. Demographic data per participant.

Gender	Age	Smoking	Allergy	Asthma
Healthy controls				
Male	29	No	No	No
Male	41	No	No	No
Female	61	No	No	No
Male	58	No	No	No
Male	51	No	No	No
Male	18	No	No	No
Male	23	No	No	No
Male	28	No	No	No
Female	19	No	No	No
Female	49	No	No	No
Female	52	No	No	No
Male	28	No	No	No
Female	39	No	No	No
Female	63	No	No	No
Female	22	No	No	No
Female	56	Yes	No	No
Male	46	No	No	No
Female	43	No	No	No
Allergic rhinitis				
Male	33	No	HDM, grass pollen, animal dander	No
Male	31	No	HDM, grass pollen	No
Female	23	No	HDM, grass pollen	No
Male	60	No	HDM, tree pollen	No
Male	28	No	HDM, grass/tree pollen, animal dander	No
Male	53	Yes	HDM, grass pollen	No
Female	38	No	HDM, grass pollen	No
Female	20	No	HDM, animal dander	Yes
Male	25	Yes	HDM, animal dander	No
Male	62	No	HDM, grass/tree pollen, animal dander	No
Male	53	No	HDM, grass/tree pollen, animal dander	No
Female	36	No	HDM, grass/tree pollen, animal dander, fungi	No

Gender	Age	Smoking	Allergy	Asthma
Male	20	No	HDM, grass pollen	No
Male	31	No	HDM, grass/tree pollen, animal dander	No
Female	58	No	HDM, animal dander	No
Female	28	No	HDM	No
Female	28	No	HDM, grass pollen, animal dander	No
Female	29	Yes	HDM, fungi	No
Male	26	Yes	HDM, animal dander	No
Female	31	No	HDM, grass pollen	No
Male	29	Yes	HDM	No
Female	44	No	HDM, grass pollen, animal dander	No
Male	41	No	HDM, grass/tree pollen, animal dander	No
CRSwNP				
Male	45	Yes	No	No
Female	58	No	No	No
Male	29	No	No	No
Female	56	No	No	No
Male	45	No	No	No
Male	33	No	No	No
Male	62	Yes	No	No
Male	60	No	No	No
Male	46	No	No	No
Male	43	No	No	No
Male	65	No	No	No
Male	56	No	No	Yes
Male	57	No	No	No
Female	40	No	No	Yes
Male	31	No	No	No
Male	60	No	No	Yes
Male	61	No	No	No
Female	65	No	No	No
Female	43	No	No	No
Male	33	Yes	No	No
Female	57	No	No	No
Male	40	No	No	No

HDM: house dust mite, AR: allergic rhinitis, CRSwNP: chronic rhinosinusitis with nasal polyps.

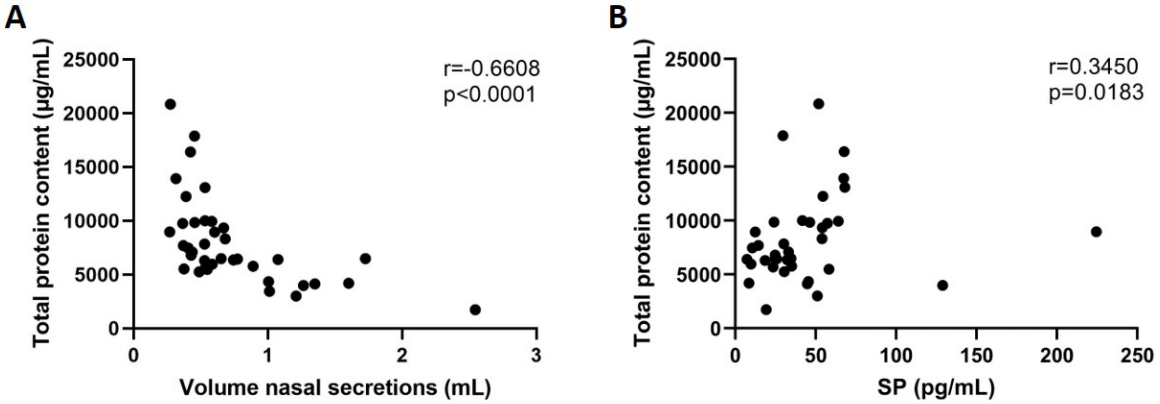

Figure S1. Correlation between total protein concentration and the collected volume of nasal secretions (A) or measured Substance P (SP) concentration (B). (Spearman r test).

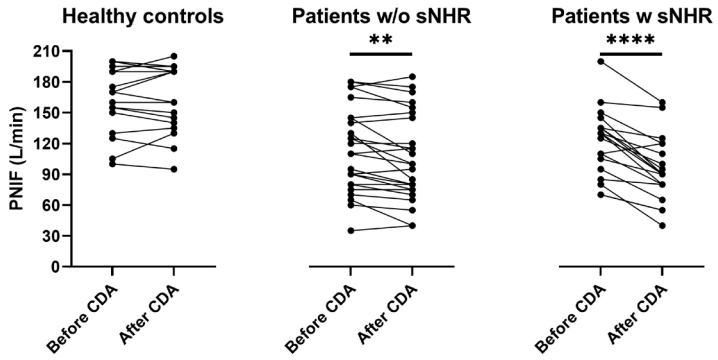

Figure S2. Individual measurements of peak nasal inspiratory flow (PNIF) before and after cold, dry air provocation test (CDA) in healthy controls and patients without or with self-reported nasal hyperreactivity according to the two-part question (sNHR). (Paired t-test, ** $p < 0.01$, **** $p < 0.0001$).

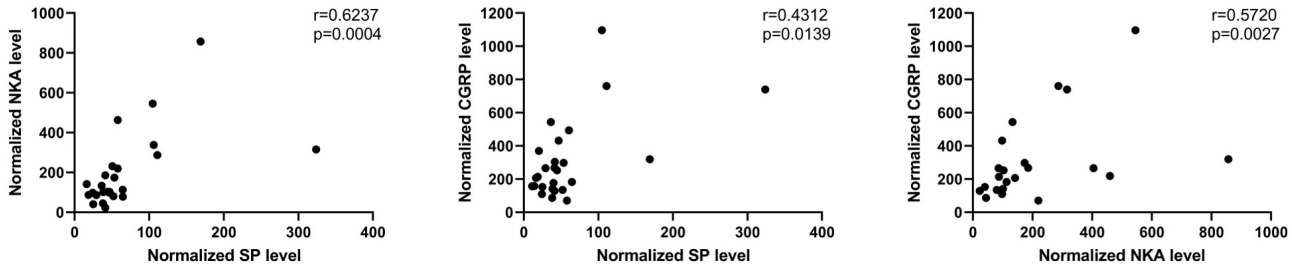

Figure S3. Correlations between the various measured neuropeptides in nasal secretions of all patients (regardless of reactivity to cold, dry air). (Spearman r test).

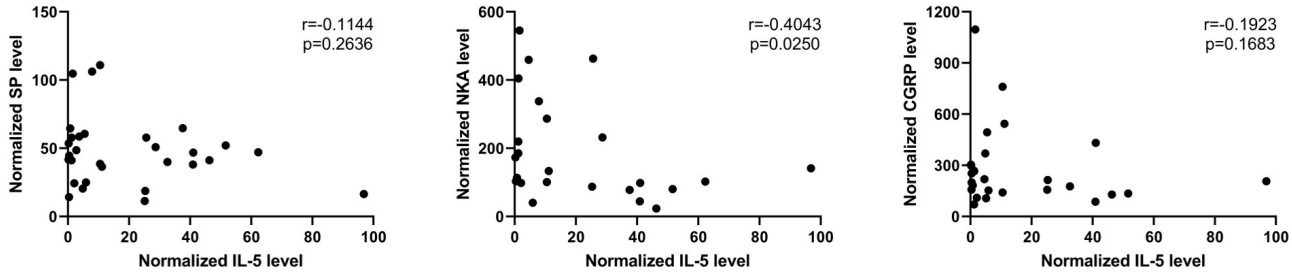

Figure S4. Correlations between level of the neuropeptides substance P (SP), neurokinin A (NKA), and calcitonin gene-related peptide (CGRP) on one side and interleukin 5 (IL-5) on the other side present in nasal secretions of all patients (regardless of reactivity to cold, dry air). (Spearman r test).

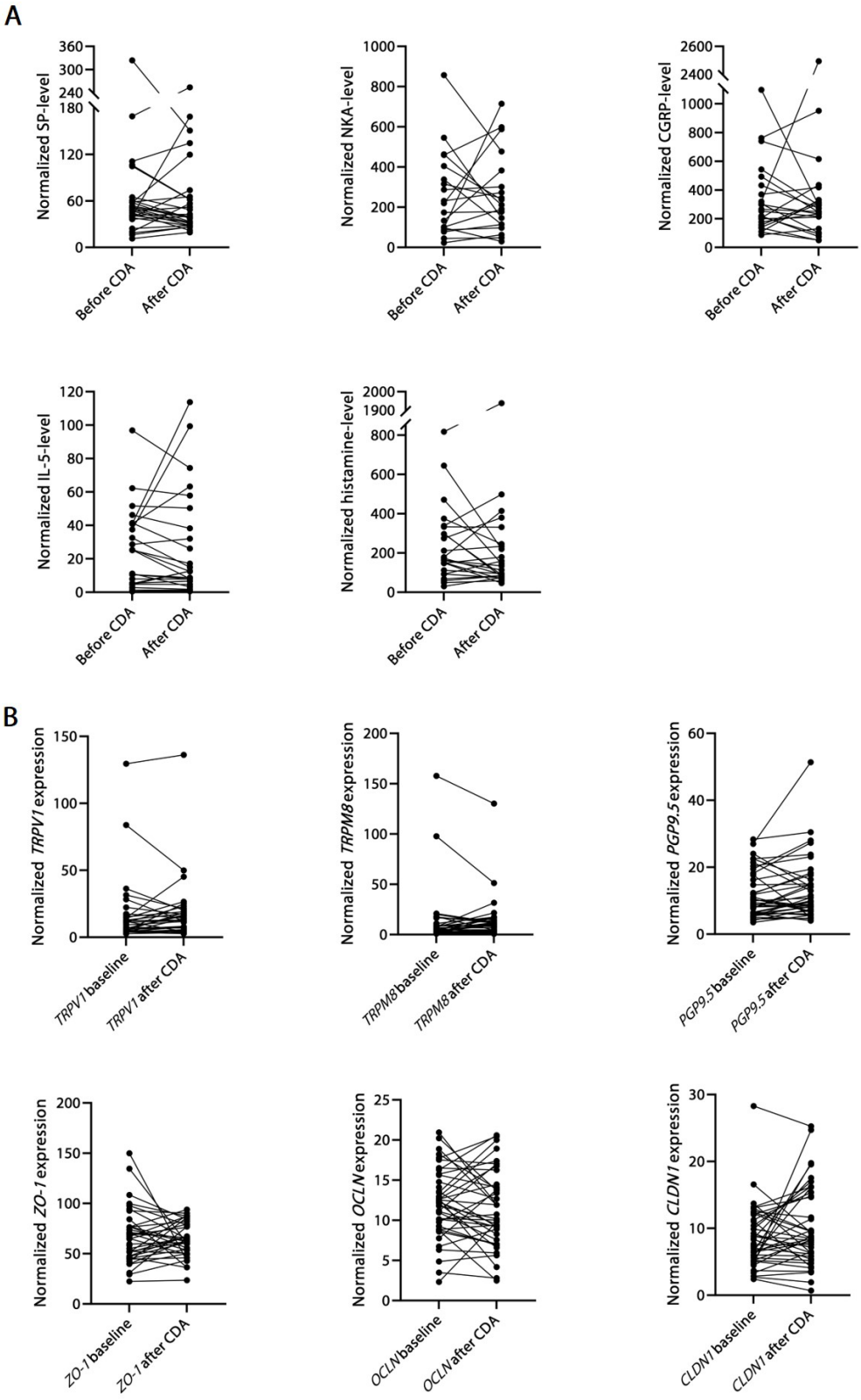

Figure S5. Normalized expression of SP-, NKA-, CGRP-, IL-5-, and histamine-levels (A) and of TRPV1, TRPM8, PGP9.5, ZO-1, OCLN, and CLDN1 mRNA in nasal mucosal biopsies (B) at baseline and after cold, dry air (CDA) provocation. (Wilcoxon matched-pairs signed rank test, not significant.) SP: substance P, NKA: neurokinin A, CGRP: calcitonin gene-related peptide, IL: interleukin, TRPV1: transient receptor potential channel vanilloid 1, TRPM8: transient receptor potential channel melastatin 8, PGP9.5: protein gene-product 9.5, ZO-1: zonula occludens 1, OCLN: occludin, CLDN1: claudin 1.

AR patients

	No oNHR	oNHR
No sNHR	8	2
sNHR	6	7

CRSwNP patients

	No oNHR	oNHR
No sNHR	14	2
sNHR	0	6

Figure S6. Clinical evaluation of subjective (sNHR) and objective nasal hyperreactivity (oNHR) in patients with allergic rhinitis (AR) or chronic rhinosinusitis with nasal polyps (CRSwNP).