



Contents lists available at ScienceDirect

International Journal of Hygiene and Environmental Health

journal homepage: www.elsevier.com/locate/ijheh



Does seasonal allergic rhinitis increase sensitivity to ammonia exposure?

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ARTICLE INFO

Article history:

Received 5 January 2017
Received in revised form 31 March 2017
Accepted 31 March 2017

Keywords:

Allergy
Chemical sensitivity
Trigeminal chemoreception
Sensory irritant

ABSTRACT

Allergic inflammation in the upper airways represents a wide-spread health issue: Little is known about whether it increases sensitivity to airborne chemicals thereby challenging established exposure limits that neglect such differences in susceptibility. To investigate the role of pre-existing allergic inflammation, 19 subjects with seasonal allergic rhinitis (SAR) and 18 control subjects with low risk of sensitization were exposed for 4 h to ammonia in two concentrations (cross-over design): 2.5 ppm (odor threshold) and 0–40 ppm (occupational exposure limit: 20 ppm TWA). Prior to the whole-body exposure, it was confirmed that subjects with SAR showed persistent inflammation outside the pollen season as indicated by increased exhaled nitric oxide and total immunoglobulin E in serum compared to controls. Despite concentration-dependent increases in chemosensory perceptions and acute symptoms, SAR status did not modulate subjective effects of exposure. Moreover, SAR status did not affect the investigated physiological endpoints of sensory irritation: While eye-blink recordings confirmed weak ocular irritation properties of ammonia at 0–40 ppm, this effect was not enhanced in SAR subjects compared to controls. Irrespective of SAR status, exposure to 0–40 ppm ammonia did not result in a cortisol stress response, objective nasal obstruction as measured with anterior active rhinomanometry, or an inflammatory response as indexed by substance P, tumor-necrosis-factor α , and high-mobility-group protein 1 in nasal lavage fluid. At least for the malodorous compound ammonia, these results do not support the hypothesis that SAR enhances chemosensory effects in response to local irritants. Before generalizing this finding, more compounds as well as sensitization to perennial allergens need to be investigated.

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1. Introduction

Seasonal allergic rhinitis (SAR) is a wide-spread health issue in Western countries which affects about 10–20% of the population in a given year (Dykewicz and Hamilos, 2010). It is mediated by the recognition of allergens such as grass pollen by specific immunoglobulin E (IgE) antibodies in sensitized individuals (type I hypersensitivity) leading to the rapid release of inflammatory

mediators such as histamine (Greiner et al., 2011). These mediators cause the typical nasal and ocular symptoms associated with SAR such as sneezing, rhinorrhoea, and eye watering.

In a recently published review, Shusterman (2014) argued that a pre-existing inflammation in the upper airways as found in individuals with SAR confers enhanced upper airway sensitivity to air pollutants. Compounds such as ammonia are major contributors to odor annoyance in the environment and at industrial workplaces (Blanes-Vidal et al., 2012; Ihrig et al., 2006). Moreover, exposure to most of these air pollutants in higher concentrations can also cause sensory irritation by activating trigeminal free nerve endings located in the nasal cavity and the eyes (Hummel and Livermore, 2002). This is associated with neurogenic reflexes (e.g. release of substance P, see Chiu et al., 2012) and increased nasal congestion (Shusterman, 2016).

In a series of studies Shusterman and colleagues showed that individuals with SAR indeed exhibit lower lateralization thresholds for irritating *n*-propanol (Shusterman, 2007; Shusterman

Abbreviations: FeNO, fraction of exhaled nitric oxide; HMGB-1, high-mobility-group protein 1; IgE, immunoglobulin E; LMS, Labeled Magnitude Scale; SAR, seasonal allergic rhinitis; SP, substance P; SPES, Swedish Performance Evaluation System; TNF- α , tumor-necrosis-factor α .

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<http://dx.doi.org/10.1016/j.ijheh.2017.03.013>

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et al., 2003a) and an augmented nasal congestive response after exposure to chlorine gas and acetic acid compared to healthy controls (Shusterman et al., 2003b, 2005, 1998). These results are underpinned by electrophysiological evidence showing that higher symptom scores in allergic rhinitis are linked to shorter latencies of event-related potentials in response to nasal CO₂ stimulation (Doerfler et al., 2006).

In line with this, about 40% of individuals with SAR report enhanced sensitivity to non-allergic triggers such as odorous and irritating chemicals (Shusterman and Murphy, 2007). A recent epidemiologic study indicates that even innocuous perfume odors may cause exaggerated reactions in individuals with SAR (Claeson et al., 2016). This suggests that individuals with SAR may be especially susceptible to occupational and environmental chemical exposures compared to individuals without SAR.

Ovalbumin (OVA) sensitized rodent models (Yiamouyiannis et al., 1999) have been used to mimic allergen-related sensitization of humans as indicated by an increased serum IgE level in these animals. Based on these animal models enhanced responses to acrolein and acetic acid could be confirmed in sensitized animals (Morris et al., 2003). However, differences between the two compounds and the two investigated endpoints were shown. For both compounds the sensory response measured as reduction in respiratory rate (RD₅₀-like readout) was generally increased in the OVA-sensitized animals. In contrast, only the airway obstructive response to acetic acid was increased in these animals. Here the duration of the sensitization which is positively associated with its severity was an important factor. Thus, these animal studies as well as the aforementioned epidemiological studies suggest that health based exposure limits for airborne chemicals need to consider inter-individual variability in SAR status (e.g. Brüning et al., 2014).

However, before such a step is taken, a wider range of compounds needs to be tested under controlled but natural breathing conditions (compare Shusterman, 2016). While Shusterman and colleagues used concentrations of chlorine gas and acetic acid that corresponded to (U.S.) short-term exposure limits in their studies (Shusterman et al., 2003b, 2005, 1998), the exposures were restricted to 15 min. Moreover, only a single physiological endpoint, namely objective nasal obstruction, was assessed. To explore the chemosensory effects of acute exposures to local irritants other physiological endpoints such as eye-blink frequency (Kiesswetter et al., 2005) or concentrations of nasal neuropeptides (van Thriel et al., 2003) might be considered more sensitive markers of relevant sensory irritation processes (Brüning et al., 2014).

Therefore, we tested the hypothesis of enhanced sensitivity to airborne chemicals in SAR during an experimental 4 h whole-body exposure to ammonia: In response to concentrations of ammonia at the occupational limit and at the odor threshold (i.e. two conditions in a cross-over design) diverse physiological and subjective effects were assessed in subjects with SAR and control subjects with a low risk of sensitization to seasonal allergens (multilevel approach, see Kleinbeck et al., 2008).

Ammonia was selected as a test substance (a) due to its ubiquitous potential for exposure in daily life (e.g. use in household cleaning products), (b) due to its psychophysically well-established odor threshold (Smeets et al., 2007) and (c) due to its strong impact on reported chemosensory perceptions and symptoms (Ihrig et al., 2006; Pacharra et al., 2016a,d). Accordingly, it was expected that ammonia would concentration dependently impact chemosensory mediated endpoints with stronger effects for SAR compared to control subjects (compare Shusterman, 2014). As in previous studies on the effects of SAR, all subjects were tested outside the pollen season (Shusterman et al., 2003b, 2005, 1998): This was done to avoid confounding effects of pre-existing acute nasal congestion.

Table 1

Descriptive data of the subjects with seasonal allergic rhinitis (SAR) and controls (means and standard deviations shown).

	controls	SAR	^a p-value
women/men, n	10/8	9/10	ns
age, years	23.6 ± 2.5	25.1 ± 3.9	ns
FEV1 (% of predicted)	98.9 ± 10.8	98.2 ± 9.7	ns
odor identification ability	12.9 ± 1.4	13.3 ± 1.6	ns
FeNO, ppb	15.3 ± 6.2	38.5 ± 31.2	.004
total immunoglobulin E, kU/l	39.9 ± 45.2	169.8 ± 137.0	.001
trait anxiety	33.8 ± 8.3	39.9 ± 9.4	.043
self-reported general sensitivity	28.1 ± 12.8	39.8 ± 17.6	.026
self-reported trigeminal mediated sensitivity	4.7 ± 5.9	7.7 ± 7.7	ns

FEV1 = volume that was exhaled during the first second of forced expiration; FeNO = fraction of exhaled nitric oxide.

^a Independent samples *t*-test. ns = not significant.

2. Material and methods

2.1. Subjects

Forty-eight non-smoking subjects were recruited for the study. Exclusion criteria were pregnancy, history of asthma and chronic diseases (in addition to SAR), current use of antihistamines, nasal steroids, and decongestants. For the purpose of this study, confirmed SAR was defined as (a) a history of seasonally occurring symptoms which are in accordance with allergic rhinitis (Shusterman et al., 2003b, 2005, 1998), (b) a report of a medical diagnosis of seasonal allergic rhinitis in the past, and (c) a concentration of allergen-specific IgE in serum consistent with reported allergens (77.3% of subjects: grass pollen; 22.7%: tree or hazel pollen) and indicative of allergic rhinitis (>.35 AU/ml, see e.g. Bokanovic et al., 2013; Hatzler et al., 2012). Subjects who reported (a) no history seasonally occurring symptoms which are in accordance with allergic rhinitis and (b) no medical diagnosis of seasonal allergic rhinitis in the past were considered at low risk for sensitization to seasonal allergens. For the purpose of this study, these subjects were considered as control subjects.

Before participating in the study, all subjects passed a health check by an occupational physician on a separate test day (Kobald et al., 2015). The standardized physiological and psychological assessment included (a) a lung function test (Vitalograph, Hamburg, Germany), (b) an olfactory function test (Sniffin' Sticks identification test, Burghart, Wedel, Germany), (c) a measurement of fractional exhaled nitric oxide (FeNO; NIOX Mino, Aerocrine, Sweden, see ATS/ERS, 2005), (d) an analysis of total IgE in serum from venous blood samples (DIN EN ISO 15189 certified laboratory), and (e) questionnaires on trait anxiety (Spielberger, 1983) and self-reported sensitivity (Kiesswetter et al., 1999). Trait anxiety and self-reported sensitivity constitute relevant inter-individual differences in chemosensory-mediated adverse effects (Pacharra et al., 2016b).

Eleven subjects were omitted from data analysis: Five subjects were excluded due to missed test days, three subjects due to technical problems during the recording of eye blinks, and three subjects due to technical problems during the nasal fluid sampling. Descriptive data for the remaining 37 subjects is presented in Table 1.

As can be seen in Table 1, the control and the SAR group did not differ with respect to gender ratio, age, lung function, odor identification ability, and self-reported trigeminal mediated sensitivity. However, subjects with SAR had higher FeNO and a higher total IgE concentration in serum compared to controls. Moreover, SAR subjects reported stronger general sensitivity and trait anxiety than controls.

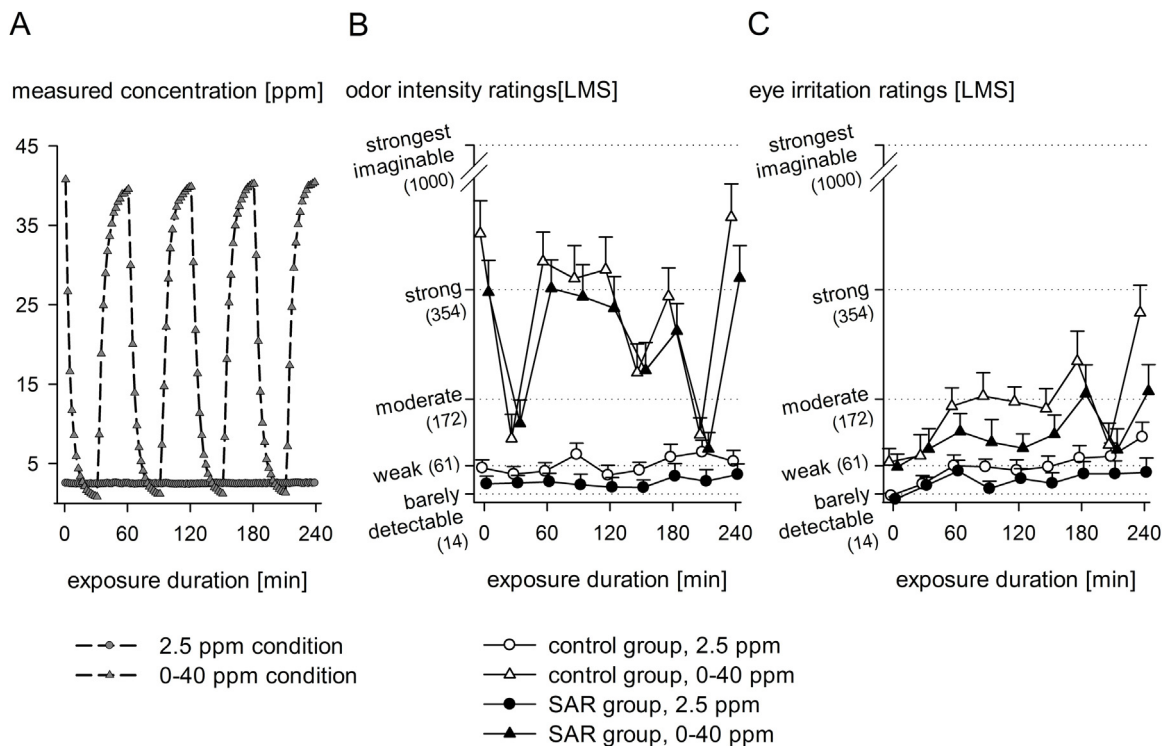


Fig. 1. Time courses of (A) mean measured concentrations, (B) odor intensity ratings (means + SEM), and (C) eye irritation ratings (means + SEM) during the 4 h exposure to 2.5 and 0–40 ppm ammonia.

All subjects were advised to refrain from alcohol, caffeine, drugs, and physical exercise on the two experimental exposure days. Acute infections were excluded by a brief examination performed by an occupational physician before each exposure session.

2.2. Procedure

The local ethics committee approved the study protocol. All subjects provided written informed consent. In the exposure laboratory, subjects were tested in groups of four subjects that were mixed with regards to gender and SAR status. On two different test days, subjects were exposed for 4 h to a constant concentration of 2.5 ppm (median odor threshold, see Smeets et al., 2007) and to a variable concentration of 0–40 ppm ammonia (time-weighted average = 20 ppm, German occupational exposure limit, see DFG, 2002). A variable exposure scenario was chosen, as exposure peaks can generally reduce chemosensory adaptation (van Thriel et al., 2005). The exposure peaks in this study (40 ppm) were in the range of the lateralization threshold of ammonia (30–60 ppm, see Smeets et al., 2007). The time courses of the ammonia concentration in the two exposure conditions are depicted in Fig. 1A.

While one half of the subjects started with the 2.5 ppm exposure on their first test day, the other half started with the 0–40 ppm exposure (cross-over design). The test days were one week apart from each other. All exposures were conducted between 09:00 a.m. and 01:00 p.m. to control for diurnal cycle of cortisol.

During exposure, chemosensory perceptions and acute symptoms were assessed approximately every 30 min (see Pacharra et al., 2016c). Eye-blinks were recorded during two 30 min intervals starting 30 min and 210 min after exposure onset (see Kiesswetter et al., 2005). Before and after exposure, physiological measurements were performed in the following order: First, saliva samples were collected. Next, nasal obstruction was assessed via rhinomanometry (see Wiesmüller et al., 2002). Afterwards, FeNO was determined (see Hoffmeyer et al., 2016) and then nasal lavage fluid was collected (see van Thriel et al., 2003).

2.3. Exposure to ammonia

The experimental whole-body exposure was conducted in a ventilated 28 m³ exposure laboratory that includes four PC work stations. A climate control unit in a neighboring room supplied the lab with conditioned air. As described in detail in previous studies (Pacharra et al., 2016a,d), a spray nozzle driven by pressurized air was used to vaporize an ammonium hydroxide solution (1:1 dilution of a solution of >25% ammonia). Thus, a predefined amount of ammonia (experimentally determined by volumetric analysis) was mixed into the inlet airstream of the climate control system. The conditioned air was dispersed throughout the entire laboratory with the help of a branched pipe system, which was located on the floor.

Every two minutes of the exposure, air samples were collected by four sampling devices which were located at the ceiling of the lab. Airborne concentration of ammonia was analyzed using photoacoustic infrared spectroscopy (INNOVA, 1412i Photoacoustic Field Gas-Monitor, LumaSense, Ballerup, Denmark). Furthermore, relative humidity and temperature in the lab were continuously recorded by a standalone data logger (EasyLog, Lascar Electronics Ltd. Uk, Whiteparish, UK). There was a tenfold air exchange per hour during all exposures.

As depicted in Fig. 1A, air monitoring results confirmed that intended concentration values were achieved in the 2.5 ppm ($M=2.5$ ppm, $SD=0.04$ ppm) and in the 0–40 ppm condition ($M=19.8$ ppm, $SD=0.4$ ppm). Average relative humidity during all exposures was 49.0% ($SD=1.5\%$) and average temperature was 23.2 °C ($SD=0.3$ °C).

2.4. Ratings of chemosensory perceptions and acute symptoms

Before the exposure, all subjects were familiarized with the standardized rating scales for chemosensory perceptions and acute symptoms which were adapted for the computer screen (see Pacharra et al., 2016c). Ratings of the following chemosensory per-

ceptions were collected with the help of Labeled Magnitude Scales (LMS) which have ratio-like properties (Green et al., 1996): disgust, annoyance, odor intensity, sneeze, prickling, tickling, burning, sharp, pungent, nasal irritation, and eye irritation. The logarithmically labeled visual analogue scales ranged from *barely detectable* to *strongest imaginable* (numeric range: 1–1000).

Subjects rated the acute symptoms of the “Acute Symptoms” subtest of the Swedish Performance Evaluation System (SPES, Iregren et al., 1996) which has been expanded to comprise acute chemosensory-mediated symptoms (Seeber et al., 2002; van Thriel et al., 2003). The severity of 29 acute symptoms was assessed using a 6-point scale which ranged from *not at all* (=0) to *very, very much* (=5). The ratings were combined to form the following seven subscales: unspecific/prenarcotic symptoms, olfactory symptoms, taste symptoms, respiratory symptoms, general irritation symptoms, nasal irritation symptoms, and ocular irritation symptoms.

2.5. Eye-blink frequency

Eye-blinks caused by contractions of the musculus orbicularis oculi were assessed by electromyographic recordings as used and described in previous studies (Kiesswetter et al., 2005; Kleinbeck et al., 2008). To standardize the visual demands (see Wolkoff et al., 2003), subjects performed a vigilance test during the two 30 min recording intervals during the 2.5 and 0–40 ppm exposure (starting 30 min and 210 min after exposure onset). For statistical analyses, only the first and last 5 min of these recordings were selected: This was done to be able to compare exposure to low and high concentrations of ammonia within the 0–40 ppm exposure scenario (0 ppm dell vs. 40 ppm peak, see Fig. 1A) as well as across the two exposure scenarios (2.5 ppm vs. 0–40 ppm).

2.6. Rhinomanometry

Using computer-aided rhinomanometry (CAR), nasal congestion was measured before and after each exposure (van Thriel et al., 2003; Wiesmüller et al., 2002). This technique assesses simultaneously nasal airflow and the transnasal pressure gradient between the nostrils and the epipharynx. Increased nasal resistance and lower nasal airflow are the consequences of nasal congestion. Left and right nostrils were measured separately.

Flow values at transnasal pressures of 75, 150, and 300 Pa were calculated from the flow curves and only values fulfilling the CAR criteria were analyzed. In accordance with established standards in rhinomanometry (Clement, 1984; Clement and Gordts, 2005), for data analyses nasal airflow at a transnasal pressure of 150 Pa was analyzed and Δ values were calculated (Δ = post measurement – pre measurement). Due to technical problems two different rhinomanometers were used in different periods of the study (18 subjects: Atmos Inc., Lenzkirch, Germany; 19 subjects: Rhino-Sys, Otopront, Hohenstein, Germany; subject groups mixed with regard to SAR).

2.7. Saliva

Saliva samples were collected using Salivette sampling devices (Sarstedt, Nümbrecht, Germany) before and after each exposure (Pacharra et al., 2016a). Until biochemical analysis took place, samples were stored at -20°C . Commercially available ELISA-kits (IBL, Hamburg, Germany) were used to analyze cortisol in saliva samples as a marker of potential stress response in SAR compared to control subjects during provocation (compare Kalogeromitros et al., 2007).

2.8. FeNO

Standardized measurement of FeNO was performed using a hand-held device (NIOX Mino, Aerocrine AG) according to established guidelines (ATS/ERS, 2005). FeNO proved to be a useful marker of airway inflammation (Hoffmeyer et al., 2016; Kalpaklioglu and Kalkan, 2012). Even off-season, FeNO values are elevated in subjects with SAR compared to subjects without SAR (Gratziou et al., 2001; Henriksen et al., 1999).

2.9. Nasal lavage fluid

For the collection of nasal lavage fluid (Pacharra et al., 2016a; van Thriel et al., 2003) 5 ml of pre-warmed phosphate-buffered-saline was inserted into each nasal cavity with the help of a sterile pipette. After keeping the phosphate-buffered-saline in the nose for 10 s, subjects let the lavage fluid flow into funneled 15 ml PP-test tubes. The volume of the recovered lavage fluid was recorded. Samples were immediately stored on ice and within the next 30 min frozen at -80°C until biochemical analysis took place.

As neuropeptide concentration in nasal lavage fluid stored at 4°C proved to be stable up to 50 h without any additives, no protease inhibitors were added (Schultz et al., 1996). Commercially available ELISA-kits were used to analyze concentrations of substance P (SP; IBL, Hamburg, Germany), tumor-necrosis-factor- α (TNF- α ; Cayman Chemical Company, Ann Arbor, USA), and high-mobility-group-protein 1 (HMGB-1; IBL, Hamburg, Germany) in the nasal lavage fluid. These three markers were selected to cover various, relevant physiological responses like (a) the release of neuropeptides from axons during neurogenic inflammation (Chiu et al., 2012), (b) the subsequent release of pro-inflammatory cytokines from mast cells (Azzolina et al., 2003), and (c) the proinflammatory cytokine HMGB-1 that was found to be associated with the severity of chronic rhinosinusitis (Min et al., 2015).

2.10. Statistics

Analyses were carried out in SPSS 24 (IBM Corp.). The level of significance for all statistical tests was set to 0.05.

Chemosensory perceptions and physiological data were analyzed using repeated-measures analyses of variance (ANOVAs) with allergy status (control/SAR) and gender (male/female) as between-subjects factors and exposure condition (2.5 ppm/0–40 ppm) and time as within-subjects factors. Symptom rating data were analyzed using corresponding generalized linear models (GLM) for ordinal response data with a probit link function. If the assumption of sphericity was violated, Greenhouse–Geisser corrected degrees of freedom were used.

Nine different time points were considered for subjective data (assessed every 30 min of 240 min exposure) and two time points for the analysis of FeNO, saliva and nasal lavage markers (assessed before and after exposure). As in previous studies (Kleinbeck et al., 2008; Pacharra et al., 2016c), the impact of exposure time on eye-blink frequency was analyzed in detail: two within-subject factors namely beginning and end of the exposure (two repetitions of the vigilance task), and time of measurement during the vigilance task (first and last 5 min periods of the 25 min task) were considered.

Regarding rhinomanometric data the factor time was omitted, as in accordance with previous studies Δ values were analyzed (see Wiesmüller et al., 2002). As due to technical problems two different rhinomanometers were used in different study periods, the between-subject factor “rhinomanometer” was included in the statistical data analysis. Moreover, rhinomanometer-specific data is presented in the Supplement.

For nasal lavage analyses, values below the detection limit (SP 12.2%, TNF-alpha 41.2%, and HMGB-1 10.8% of all values) were

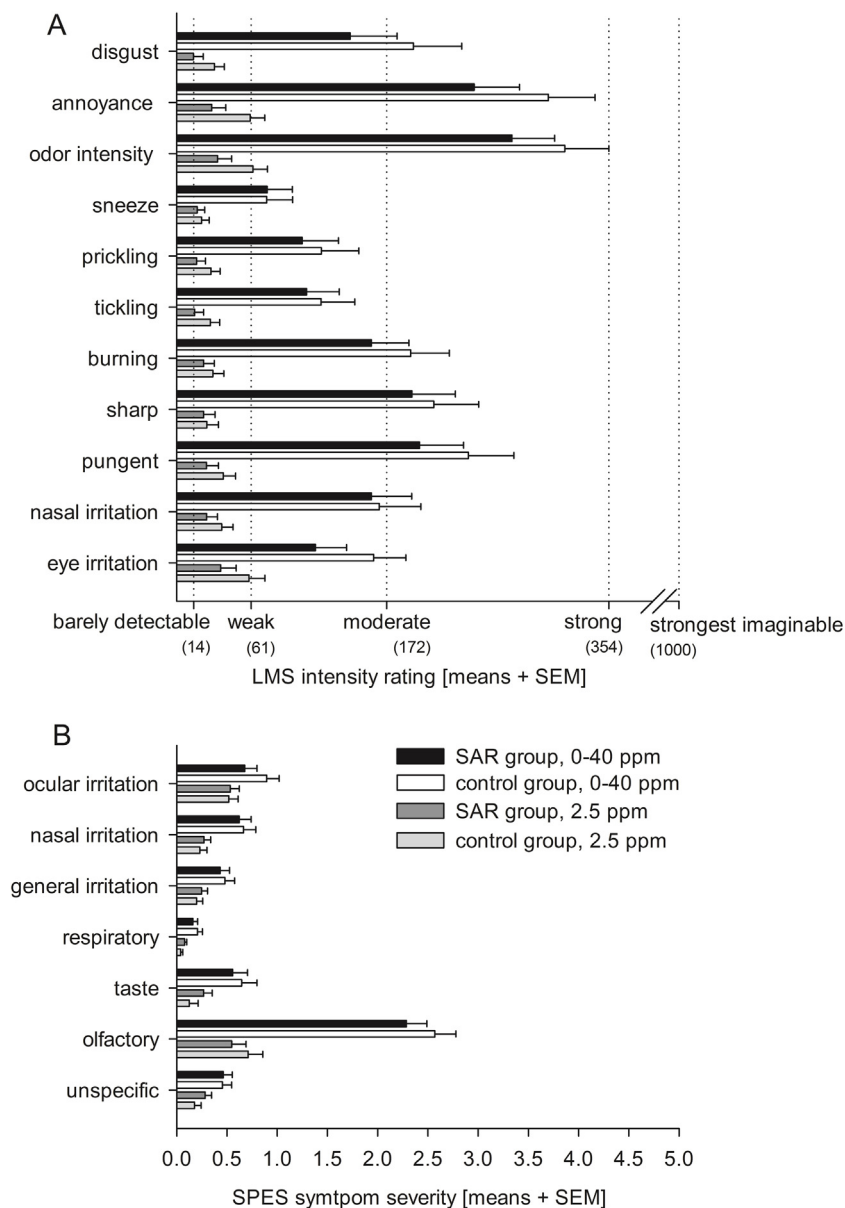


Fig. 2. Mean ratings of (A) chemosensory perceptions and of (B) acute symptoms during the 4 h exposure to 2.5 and 0–40 ppm ammonia by control and SAR subjects.

substituted with the detection limit of the assay (SP = 1.5 pg/ml, TNF- α = 0.13 pg/ml, HMGB-1 = 1.0 ng/ml) (see Pacharra et al., 2016a).

3. Results

3.1. Ratings of chemosensory perceptions and acute symptoms

Fig. 2 depicts the average ratings for the eleven chemosensory perceptions (A) and the seven symptom subscales of the SPES (B) during the two exposure conditions.

ANOVA results show that there was a significant main effect of exposure condition on all LMS ratings with higher ratings during the 0–40 ppm compared to 2.5 ppm condition (compare Fig. 2A). With the exception of ratings for sneezing, there was a significant main effect of exposure time on all LMS ratings. This effect was further modulated by the exposure condition (Exposure condition $\times$ Time, $p \leq .05$). Exemplary time courses are depicted for the LMS ratings of odor intensity and eye irritation in Fig. 1B and C,

respectively. Visual inspection indicates that the measured concentration values (Fig. 1A) show a better fit to the ratings of odor intensity (Fig. 1B) than to the ratings of eye irritation (Fig. 1C).

Overall, the factor allergy status had no substantial effect on the LMS ratings as reflected by non-significant main effects of allergy status and non-significant interaction effects between allergy status and the other factors. Only one significant two-way interaction effect on the LMS rating for sneezing (Allergy status $\times$ Time, $F_{5,167} = 2.7$, $p = .018$) emerged. However, this effect could not be meaningfully interpreted, as Bonferroni-adjusted post-hoc tests revealed no significant differences between subjects with SAR and control subjects.

The inspection of Fig. 2B shows that with the exception of olfactory symptoms almost no acute symptoms were reported by the subjects. Therefore, the quantitative analysis was restricted to olfactory symptoms (compare Kleinbeck et al., 2008). The GLM results confirm that olfactory symptoms were higher in the 0–40 ppm compared to the 2.5 ppm condition as indicated by a significant effect of exposure concentration. Moreover, similarly to

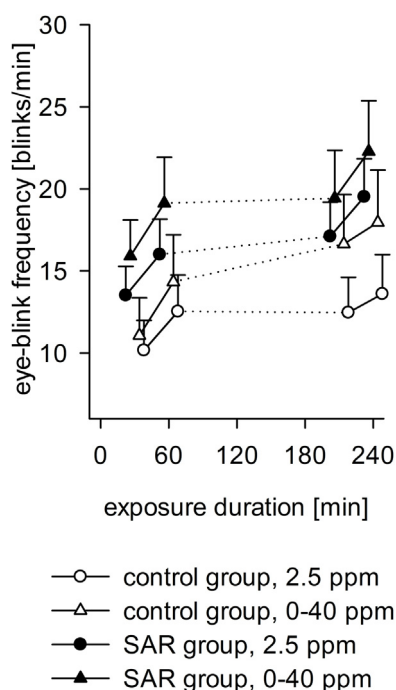


Fig. 3. Time course of the eye-blink frequency of control subjects and subjects with SAR during the 4 h exposures to 2.5 ppm (dots) and 0–40 ppm (triangles) ammonia (means + SEM).

the LMS results, olfactory symptoms were subjected to a significant main effect of exposure time and a significant interaction effect between exposure time and exposure concentration. The factor allergy status had no effect on olfactory symptoms (non-significant main effect and interaction effects with this factor).

3.2. Eye-blink frequency

A significant main effect of exposure condition on eye-blink frequency emerged ($F_{1,33} = 7.4$, $p = .010$) with higher eye-blink frequencies during the 0–40 ppm compared to the 2.5 ppm exposure (see Fig. 3). Additionally, there was a significant main effect of exposure time ($F_{1,33} = 24.9$, $p < .001$) with higher eye-blink frequencies during the second vigilance task (210–240 min after exposure onset) compared to the first vigilance task (30–60 min after exposure onset). Time of measurement also had a significant impact with higher eye-blink frequencies during the last 5 min compared to the first 5 min of the vigilance task ($F_{1,33} = 416.5$, $p < .001$).

There was no significant influence of allergy status on eye-blink frequencies as reflected by a non-significant main effect and non-significant interaction effects between allergy status and the other factors.

3.3. Rhinomanometry

The nasal congestive response with respect to exposure condition and allergy status is depicted in Fig. 4. The repeated-measures ANOVA revealed no significant influence of the exposure condition and no significant difference between controls and SAR subjects, as well as a non-significant interaction effect between allergy status and exposure condition.

Moreover, the between-subject factor rhinomanometer and all interaction effects with this factor were not significant suggesting that the use of two different rhinomanometers in different study periods did not distort the results. Supplement Fig. 1 shows the nasal congestive response in both groups and exposure conditions

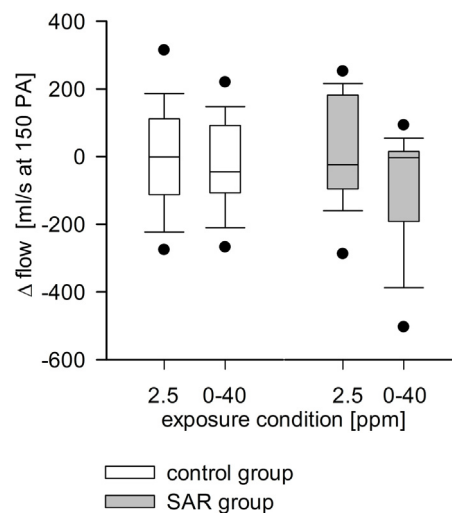


Fig. 4. Nasal congestive response after 4 h exposure to 2.5 and 0–40 ppm ammonia by control and SAR subjects. Upper and lower borders of the boxes indicate the 75th and 25th percentiles. The band near the middle of each box is the median. Whiskers above and below each box indicate the 90th and 10th percentiles. Single observations (outliers) that are located below the 10th percentile or above the 90th percentile are indicated as dots.

separately for each rhinomanometer (study part 1, $n = 18$; study part 2, $n = 19$).

3.4. Saliva

Following its diurnal cycle, salivary cortisol decreased from the beginning of each exposure session to its end (see Table 2). This was reflected by a significant main effect of the factor exposure time ($F_{1,33} = 149.1$, $p < .001$). Exposure condition and allergy status did not modulate this effect as reflected by non-significant main effects of these factors and non-significant interaction effects.

3.5. FeNO

Subjects with SAR had significantly higher FeNO compared to controls at all time points (see Table 2). This was reflected by a significant main effect of the factor allergy status ($F_{1,33} = 10.5$, $p = .003$). A non-significant main effect of the factor exposure condition and non-significant interaction effects between the factor exposure condition with the other factors indicated that the ammonia exposure had no effect on FeNO.

3.6. Nasal lavage fluid

The results of the nasal lavage analyses are shown in Table 2. Neither the exposure condition nor allergy status had an effect on TNF- α and HMGB-1 concentration in the nasal lavage fluid, as reflected by non-significant main and interaction effects. A main effect of exposure time on SP concentration emerged with higher nasal SP concentration after compared to before the exposure ($F_{1,33} = 7.1$, $p = .012$). However, this effect was neither specific to one of the exposure conditions nor to one of the experimental groups as indicated by non-significant interaction effects of exposure time with these factors.

4. Discussion

In contrast to our hypothesis, the results show that SAR does not confer increased sensitivity to acute ammonia exposures. Neither perceptions and symptoms nor physiological responses to acute

Table 2Overview of the analyses in saliva, exhaled breath, and nasal lavage fluid (means $\pm$ SEM) collected before and after the experimental exposure to 2.5 and 0–40 ppm ammonia.

		2.5 ppm		0–40 ppm	
		pre	post	pre	post
cortisol, nmol/l	control	46.6 $\pm$ 6.2	8.7 $\pm$ 1.1	49.6 $\pm$ 5.9	9.2 $\pm$ 0.8
	SAR	47.4 $\pm$ 4.4	7.7 $\pm$ 0.6	57.0 $\pm$ 6.8	8.4 $\pm$ 0.7
FeNO, ppb	control	16.8 $\pm$ 1.9	16.7 $\pm$ 1.6	16.1 $\pm$ 1.9	16.3 $\pm$ 2.1
	SAR	39.1 $\pm$ 6.7	40.3 $\pm$ 6.9	40.2 $\pm$ 6.8	40.6 $\pm$ 6.9
SP, pg/ml	control	5.4 $\pm$ 1.0	9.3 $\pm$ 1.9	7.5 $\pm$ 1.6	7.2 $\pm$ 1.2
	SAR	5.3 $\pm$ 0.8	6.8 $\pm$ 0.9	5.1 $\pm$ 1.1	6.5 $\pm$ 1.4
TNF- α , pg/ml	control	0.4 $\pm$ 0.1	0.4 $\pm$ 0.1	0.5 $\pm$ 0.1	0.5 $\pm$ 0.1
	SAR	0.3 $\pm$ 0.1	0.3 $\pm$ 0.3	0.6 $\pm$ 0.2	0.8 $\pm$ 0.4
HMGB-1, ng/ml	control	2.9 $\pm$ 0.6	2.2 $\pm$ 0.4	3.1 $\pm$ 0.7	2.3 $\pm$ 0.7
	SAR	2.6 $\pm$ 0.7	3.0 $\pm$ 1.1	3.2 $\pm$ 0.6	3.0 $\pm$ 0.6

FeNO = fractional exhaled nitric oxide; SP = substance P; TNF- α = tumor-necrosis-factor α ; HMGB-1 = high-mobility-group protein 1.

ammonia exposure differed between SAR and control subjects. With some limitations, this has important implications for regulatory decision-making in environmental and occupational contexts: It could indicate that established health based exposure limits might also adequately protect individuals with SAR from adverse effects (compare Br uning et al., 2014).

Ammonia is a confirmed sensory irritant with a lateralization threshold between 30 and 60 ppm (Smeets et al., 2007) and it acts via the TRPV1 ion channel (Dhaka et al., 2009). Compared to other occupationally regulated chemicals such as ethyl acetate or propionic acid (Kleinbeck et al., 2008; Pacharra et al., 2016c) ammonia at the occupational exposure limit of up to 40 ppm induced strong odor annoyance in this study: Subjects reported strong odor intensity and moderate trigeminal perceptions (e.g. pungency) (see Fig. 2).

The absence of a group difference between controls and SAR subjects in these subjective endpoints contrasts sharply with differences in self-reports when subjects are not acutely exposed to an airborne chemical: Self-reported sensitivity and anxiety, which are known to boost chemosensory ratings during acute chemical exposures (Pacharra et al., 2016b), were elevated in SAR subjects compared to controls (see Table 1). This is line with recent epidemiological evidence suggesting enhanced self-reported sensitivity to perfume odors in SAR (Claeson et al., 2016). Moreover, 35% of individuals with SAR specifically report cleaning products which often contain ammonia as non-allergic symptom triggers (Shusterman and Murphy, 2007).

The physiological baseline results of this study further highlight the mismatch between increased self-reported sensitivity in SAR and the observed results during acute exposure. On the one hand, olfactory ability and nasal peptides were similar in both groups at baseline suggesting the absence of acute or chronic nasal inflammation in the SAR subjects (Min et al., 2015; Salpietro et al., 2013). On the other hand, FeNO results indicated that throughout the course of the whole study SAR subjects had increased inflammation in the upper inflammatory tract compared to controls (see Table 2). Previous experimental reports identified an augmented nasal congestive response to airborne chemicals as an indicator of increased susceptibility in SAR (Shusterman et al., 2003b, 2005, 1998). But neither the nasal inflammatory marker (e.i. TNF α), the neurogenic reflex marker (e.i. substance P), nor the bronchial inflammatory markers (FeNO) in this study were differentially sensitive to ammonia exposure in the SAR compared to control subjects.

Two factors related to the experimental exposures could explain these conflicting results: The enhanced sensitivity to acute chemical exposure in SAR could be either (a) substance-specific (Morris et al., 2003; Shusterman, 2016) or (b) only relevant if the substance concentration elicits certain inflammatory processes in the upper or lower respiratory tract (e.g. 2-ethylhexanol, van Thriel et al., 2007). Airborne chemicals differ in their preferred target site in

the respiratory tract and their chemosensory potency due to their physicochemical properties (Shusterman, 2011; van Thriel et al., 2006).

In this study ammonia at the occupational limit (20 ppm time weighted average) elicited weak sensory irritation without causing physiological responses confirming the transition from sensory effects to neurogenic inflammation. This in line with a previous study which exposed healthy subjects to a constant concentration of 25 ppm ammonia for 3 h (Sundblad et al., 2004): The authors reported that exposure did not affect lung function, exhaled NO levels, IL-8 concentration in nasal lavage fluid blood leucocyte concentration. Concerning ocular irritation, Hoffmann et al. (2004) suggested that even exposures to 50 ppm ammonia do not lead to ocular irritation with only 9% of subjects showing objective ocular irritation effects after exposure. In contrast, this study indicated that weak ocular irritation occurs already during 4 h exposure at 0–40 ppm (time-weighted average: 20 ppm). The performed online assessment of eye-blink frequency is very sensitive having captured in this and previous studies increases in eye-blink frequency even during the 4 h odor control exposure (Kiesswetter et al., 2005; Kleinbeck et al., 2008; Pacharra et al., 2016c) that are most likely due to the climate control system (e.g. air exchange rate). Previous studies showed effects of SAR on lateralization thresholds for *n*-propanol (Shusterman, 2007; Shusterman et al., 2003a). Accordingly, higher concentration of ammonia might be needed to confirm these observations for this particular compound.

Interestingly, 5–30 s ammonia exposure to 100 ppm leads to a congestive response that is not different for atopic and non-atopic individuals (McLean et al., 1979). This highlights that ammonia may not be able to elicit a differential response in SAR and control subjects even at a more potent concentration. Together with our results, this suggests that the effects of SAR status on sensitivity to chemical exposure could indeed depend upon the specific chemical under investigation. This underscores recent calls that a wider range of provocation agents and various subsets of susceptible individuals need to be tested (Shusterman, 2016): For example, sensitization to prenasal allergens is common and was not specifically controlled in this study. Moreover, epidemiological studies have shown an even greater overlap between specific self-reported sensitivities and allergies (Claeson et al., 2016) than observed in this study (e.g. self-reported trigeminal mediated sensitivity, see Table 1). That indicates that more sensitive allergy sufferers than those investigated here may exist.

5. Conclusions

We could show that pre-existing inflammation as found in individuals with SAR does not confer an increased sensitivity to an airborne chemical during acute exposure. However, the chemical class as well as the concentration needs to be considered

before drawing general conclusions. Thus, at least regarding the wide-spread airborne pollutant ammonia, established health based exposure limits of about 20 ppm adequately protect individuals with and without SAR from adverse health effects.

Acknowledgements

This research was supported in part by the DGUV – German Social Accident Insurance, Berlin, Germany (FF-FP0326). The study sponsor had no influence in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the paper for publication.

The authors would like to thank Nicola Schmidt-Peucker, Eva Strzelec, Michael Porta, Gabriele Baumhoer, and Beate Aust for technical support. We are also grateful to Professor Monika Raulf for helpful advice concerning the selection of subjects with seasonal allergic rhinitis.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ijheh.2017.03.013>.

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Supplement Fig 1. Nasal congestive response after 4 h exposure to 2.5 and 0- 40 ppm ammonia by control and SAR subjects in study part 1 (n = 18) and study part 2 (n = 19). Upper and lower borders of the boxes indicate the 75th and 25th percentiles. The band near the middle of each box is the median. Whiskers above and below each box indicate the 90th and 10th percentiles. Single observations (outliers) that are located below the 10th percentile or above the 90th percentile are indicated as dots.

