Subjective Olfactory Desensitization and Recovery in Humans

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Abstract

Adaptation to smells is a well-known phenomenon and appears to be one of the major characteristics of olfaction. However, no standardized protocols for the psychophysical measurement of olfactory adaptation and recovery are available to date. Twenty normosmic participants were included. Hydrogen sulfide (H₂S) and phenylethyl alcohol were used in different concentrations based on air dilution olfactometry. Volunteers were exposed to a constant flow of odorous air until perception disappeared completely. For testing recovery, the volunteers were exposed to a reference stimulus following complete self-desensitization with the same odor. The subjects were then again exposed to the same odorant after recovery periods of different lengths and instructed to rate stimulus intensity. The time to complete desensitization increased with increasing stimulus concentration for both odorants. Subjects desensitized more rapidly using H₂S. Olfactory adaptation led to a reduction in stimulus intensity for the subsequent identical stimulation. Longer recovery periods resulted in increased intensity of the subsequent stimulus independent of the stimulus used. The results confirm current knowledge regarding the dynamics of olfactory adaptation and demonstrate differences in olfactory desensitization between the 2 odorants used. Olfactory recovery was independent of the odorant used, indicating that olfactory recovery after complete desensitization may be a uniform process.

Key words: adaptation, desensitization, olfaction, recovery

Introduction

Adaptation to odors is a well-known phenomenon in humans that is believed to be one of the major characteristics of olfaction. It describes the reduced perception of an odor as a result of constant or repetitive exposure, and it serves to facilitate the perception of new or changing olfactory stimuli. Olfactory adaptation takes place on a peripheral and a cortical level, the latter termed habituation (Thompson and Spencer 1966). Olfactory adaptation is usually described as the reduction in neural response occurring during repetitive stimulation. The decrease in neural response over time during constant stimulation is often labeled desensitization. Olfactory adaptation is mediated by Ca²⁺ in several ways (Matthews and Reisert 2003): stimulation of olfactory cilia leads to an influx in intracellular Ca²⁺, which serves as a negative feedback mechanism by, for example, reducing the activity of adenylyl cyclase (Wei et al. 1998) and by down-regulating the affinity of the cyclic nucleotide-gated (CNG) channel to adenosine monophosphate (Waldeck et al. 2009; Munger et al. 2001). A detailed overview has been provided by Zufall and Leinders-Zufall (2000).

Olfactory adaptation reduces perceived intensity of an odor and increases detection threshold. Moreover, it increases reaction time and decreases behavioral response. A detailed overview of the principles of subjective olfactory adaptation is given by Dalton (2000). The extent of olfactory adaptation moreover depends on a number of factors. These factors also have been described in behavioral experiments with drosophila larvae (Wuttke and Tompkins 2000). The larva’s behavioral reaction to an attracting olfactory substance is reduced after previous olfactory adaptation and depends on the concentration of the substance used for adaptation, the duration of adaptation, and the similarity of the odorant used for adaptation and subsequent stimulation. These principles of olfactory adaptation have been confirmed in
humans (Jacob et al. 2003). The degree of olfactory adaptation also is related to the similarity of the odors. A higher degree of adaptation is achieved when the substances used for adaptation and testing are identical. When 2 different substances are used, the degree of olfactory adaptation is related to both perceptual and structural similarity, the latter regardless of perception (Pierce et al. 1995, 1996).

Various authors have demonstrated that human olfactory adaptation further depends on the relevance of the substance (Dalton 2000). Subjects adapt to a lesser extent when they believe that the odor is important. Kobayashi et al. (2008) demonstrated that odors were perceived more intensely if the subject believed the odor to be hazardous. This difference could be demonstrated with intermittent but not constant presentation (Kobayashi et al. 2008). Finally, hedonicity seems to influence olfactory adaptation (Jacob et al. 2003). The influence of context and subjective assessment on olfactory adaptation is reflected in the cortical level of olfactory adaptation/habituation in humans.

Smith et al. (2010) recently presented a self-adaptation protocol to measure the olfactory threshold for a substance presented simultaneously with the adapting stimulus. With this protocol, the authors were able to assess the time course of rapid olfactory adaptation, which occurred milliseconds after the onset of stimulation and which relates to the time course of peripheral olfactory adaptation on a receptor level. In contrast, subjects exposed to an odorant over 2 weeks demonstrated a long-lasting reduction in sensitivity and perception of the odor that lasted up to 2 weeks after the end of adaptation (Dalton and Wysocki 1996).

It has been demonstrated on a behavioral level in drosophilae larvae that the response to a subsequent stimulus after termination of olfactory adaptation depends on the time interval between the end of exposure and subsequent (recurrent) stimulation (Wuttke and Tompkins 2000). Data on subjective olfactory recovery in humans are lacking to date.

No standardized protocols are available to date for measurement of human adaptation and olfactory recovery, especially with regard to the capacity for olfactory adaptation of different odors. In 1886, protocols for subjective olfactory adaptation (and recovery) were published by Aaronsohn (1886), demonstrating relatively rapid subjective olfactory adaptation to various odorants. The results, however, were based on subjects sniffing these substances, and therefore the stimulus conditions were relatively uncontrolled.

Standardized protocols, however, are essential for reproducible testing of olfactory adaptation and recovery. They would enable future research of these 2 basic mechanisms of olfaction, for example, to assess the impact of olfactory disorders such as hyposmia or parosmia on olfactory adaptation or to evaluate potential differences in “peripheral” and “central” olfactory disorders with regard to their impact on adaptation. Furthermore, data on olfactory adaptation and recovery are crucial in olfactory testing, for example, to define recovery periods between olfactory stimulation in electrophysiological testing.

The aim of our study was to present and evaluate 2 different protocols to measure subjective olfactory adaptation (desensitization) and olfactory recovery in humans and to quantify olfactory desensitization (and recovery) to 2 different odors.

Material and methods

The study was conducted at the Department of Otorhinolaryngology, Head and Neck Surgery, Mannheim, Germany. The study protocol was approved by the local ethics board of the Medical Faculty Mannheim at the University of Heidelberg (2011-273N-MA). Written informed consent was obtained from all participants. The study complies with the Declaration of Helsinki for medical research involving human subjects.

Two protocols were tested to quantify subjective olfactory desensitization and olfactory recovery in humans. The 2 protocols were labeled as follows:

- Protocol 1 (olfactory desensitization): Time to complete adaptation during constant olfactory stimulation.
- Protocol 2 (olfactory recovery): Impact of olfactory adaptation on subsequent olfactory stimulation, depending on the interval between adaptation and subsequent stimulation.

Participants

Twenty healthy, young normosmic volunteers of both sexes (10 female, 10 male) were included. The following inclusion and exclusion criteria were applied:

Inclusion criteria:

- Age between 18 and 30 years.

Exclusion criteria:

- History of significant smell or taste disorders.
- Current use of medication known to affect chemosensory function.
- Hyposmia or anosmia in psychophysical olfactory testing (Sniffin’ Sticks, see following).
- Relevant nasal pathologies such as mucosal inflammation, significant septal deviation, and nasal polyposis (clinical examination including nasal endoscopy).
- Relevant nasal obstruction as assessed with active anterior rhinomanometry (Rhinomanometer 300, ATMOS Medizintechnik GmbH & Co. KG).

Psychophysical testing

All participants’ olfactory function was tested using a Sniffin’ Sticks test kit (Kobal et al. 2000). Testing involved
assessment of n-butanol odor threshold, odor discrimination, and odor identification as previously described. In order to quantify olfactory function, the sum of the scores for odor threshold, odor discrimination, and odor identification was used (threshold discrimination identification [TDI] score) (Wolfensberger et al. 2000). The maximum score was 48. Normosmia was defined as a score >30.

Olfactory stimulation

A dynamic olfactometer based on air dilution olfactometry was used for stimulation (OM6b; Burghart Instruments) as previously described (Stuck et al. 2006). This instrument presents a continuous airstream of odorous stimuli (8 L/min) without producing mechanical or thermal sensations (Kobal and Hummel 1988). The subjects were advised to breathe regularly and slowly through their open mouth. Breathing patterns, however, were not verified in real time so hedonic-specific changes in nasal airflow and ensuing effects cannot be completely excluded. Both the airstream delivered by the olfactometer and the breathing patterns of the subjects, however, minimize the influence of breathing on stimulus presentation.

Two substances were used in different concentrations: hydrogen sulfide (H2S, which smells like rotten eggs) and phenylethyl alcohol (PEA, which smells like roses). Both odorants are pure olfactory stimuli with little or no trigeminal activity, they are volatile, and they are easy to use with air dilution olfactometry. In addition, they are nontoxic in nasal activity, they are volatile, and they are easy to use with air dilution olfactometry. The concentrations used and have frequently been used in olfactory research, so, for example, perception thresholds are established.

H2S was used in the following concentrations: 1, 2, 4, and 8 parts per million (ppm), all of which were clearly above threshold. PEA was used in concentrations of 10%, 20%, and 40% v/v, and again all stimuli were clearly above threshold. The concentrations and the concrete settings used for stimulation in the 2 different protocols are described in the following section. Subjects were tested multiple times (see following). If more than 1 test was performed per subject per day, time intervals of at least 10 min between tests were implemented to avoid carry over or longer lasting adaptation effects. In addition, the order of study conditions (odorant, concentrations, recovery periods) was randomized across subjects.

Protocol 1 (olfactory desensitization): Time to complete adaptation with constant olfactory stimulation

In this protocol, 10 volunteers were exposed to a constant flow of the odorant using the following concentrations: 1, 2, 4, and 8 ppm for H2S and 5%, 10%, and 20% v/v for PEA. The subjects were asked to indicate when the perception of the odor disappeared completely. The time to complete olfactory desensitization was documented for every subject and for every concentration of both odorants. Each of the 10 subjects was tested 3 times for every odorant and every concentration, resulting in 30 measurements per concentration of both odorants. The stimulation protocol is shown in Figure 1. Mean values and standard deviation (SD) of the time to complete desensitization in seconds were calculated using all 30 measurements and provided for every concentration of both substances.

Protocol 2 (olfactory recovery): Impact of olfactory adaptation on subsequent olfactory stimulation, depending on the interval between adaptation and subsequent stimulation

In this protocol, the volunteers were exposed to a reference stimulus for 500 ms (either H2S or PEA). After a period of 10 s, the subjects were then exposed to a constant flow of the same odorant in the same concentration over a set time period, resulting in complete desensitization for the majority of subjects. The set time period was based on the results of the experiments in protocol 1: 120 s for H2S and 300 s for PEA. Following this period of adaptation, the subjects were exposed again to the same reference odorant after interstimulus intervals (recovery periods) of different lengths (5, 20, 80, 180 s). The subjects were asked to rate the intensity of this odorant in comparison to the reference stimulus using a visual analog scale from 0 to 10 (0 = no perception; 10 = same intensity as reference stimulus). The protocol was applied for 3 concentrations of H2S (2, 4, and 8 ppm) and 2 concentrations of PEA (10% and 20%). Each subject was tested twice with every odorant, concentration, and recovery period, resulting in a total of 400 measurements (20 measurements per condition). The different stimulus conditions are provided in Table 1. The stimulation protocol is demonstrated in Figure 2. Mean values and SD of the subjective intensity ratings were calculated using all 20 measurements and provided for every recovery period and every concentration of both substances.

Figure 1 Stimulation protocol (protocol 1) for 4 ppm of H2S. Protocol for constant stimulation with the odorant (in this example, 4 ppm of H2S): The subjects were asked to indicate when the perception of the odor disappeared completely, which is defined as the time to adaptation. This figure is reproduced in color in the online version of the issue.
The data was analyzed using SPSS 19.0 (SPSS Inc.) and submitted to multivariate analysis of variance (MANOVA) for repeated measures. Bonferroni tests were used for post hoc comparisons. Pearson statistics were used for correlation analyses. The α level was set at 0.05. In addition, the steepness of the linear regression was calculated individually for the relation between the time to adaptation and increasing stimulus concentrations in protocol 1; these data were then compared using an ANOVA for repeated measurements with “substance” as factor.

Results

Twenty subjects were recruited and all could be included in the study (10 female, 10 male; mean age 23.5 ± 2.4 years) All subjects were normosmic (mean TDI score 37.7 ± 1.6). No adverse events occurred during the study.

Protocol 1: Time to complete adaptation during constant olfactory stimulation (olfactory desensitization)

The time to complete desensitization increased with increasing stimulus concentration for both substances and the stimulus concentration had a statistically significant influence on the time to complete desensitization in the MANOVA (H₂S: F(3,87) = 14.2, P < 0.001; PEA: F(2,58) = 22.7, P < 0.001). The differences in the time period between the concentrations were statistically significant in the Bonferroni post hoc test when comparing the 2 lower concentrations of H₂S to the 2 higher concentrations of H₂S (1 and 2 vs. 4 and 8 ppm: P < 0.05) but not when comparing the time periods between the 2 lower or between the 2 higher concentrations (1 vs. 2 ppm or 4 vs. 8 ppm). There were statistical differences between all 3 concentrations of PEA in the Bonferroni post hoc test (all P < 0.01). Detailed results are given in Tables 2 and 3 as well as in Figure 3A, B.

| Table 2 Time to complete desensitization for H₂S |
|-----------------------------|-----------------|--------|
| Concentration (ppm) | Time (s) | P value  |
| 1 | 39.5 ± 20.5 |  |
| 2 | 42.4 ± 18.8 | n.s. (2 vs. 1) |
| 4 | 57 ± 27.6 | P < 0.05 (4 vs. 2/4 vs. 1) |
| 8 | 69.9 ± 40.2 | n.s./P < 0.05/P < 0.05 (8 vs. 4/8 vs. 2/8 vs. 1) |

n.s., not significant.

| Table 3 Time to complete desensitization for PEA |
|-----------------------------|-----------------|--------|
| Concentration (% v/v) | Time (s) | P value  |
| 5 | 130.8 ± 76.3 |  |
| 10 | 165.8 ± 76.1 | P < 0.01 (10 vs. 5) |
| 20 | 231.4 ± 122.1 | P < 0.01/P < 0.01 (20 vs. 10/20 vs. 5) |
For both PEA and H$_2$S, there was a linear increase in the time to complete adaptation with increasing stimulus concentration. The corresponding function could be defined as $y = 4.5x + 35.5$ for H$_2$S and $y = 6.7x + 97.9$ for PEA, although this data has to be interpreted with care, as the number of data points available is relatively small for the calculation of such a function. Results from the analysis of the steepness of the linear regression calculated individually were nonsignificant, indicating that the factor “substance” had no significant effect on the steepness of this relation.

Protocol 2: Impact of olfactory adaptation on subsequent olfactory stimulation, depending on the interval between adaptation and subsequent stimulation (olfactory recovery)

As expected, olfactory adaptation led to a reduction in subjective stimulus intensity for subsequent identical stimulation. Compared with the reference stimulus presented before adaptation, subsequent stimulation was rated as less intense.

The differences in stimulus intensity between the reference stimulus and the subsequent stimuli were statistically significant for both odorants in relation to the length of the recovery period (H$_2$S: $F(3,48) = 26.2$, $P < 0.001$; PEA: $F(3,57) = 15.4$, $P < 0.001$), but they were only statistically significant for PEA in relation to the concentration of the adaptive stimulus (H$_2$S: $F(2,32) = 0.71$, $P = 0.50$; PEA: $F(1,19) = 5.97$, $P = 0.025$).

Subjective intensity of the subsequent stimulus was dependent on the length of the recovery period: longer recovery periods led to increased perception/subjective intensity of the subsequent stimulus. The differences in stimulus intensity between the different recovery periods were statistically significant for H$_2$S (5 vs. 80 s/180 s: $P < 0.001$; 20 vs. 80 s/180 s: $P < 0.001$). A similar effect was found for PEA (5 vs. 80 s/180 s: $P < 0.01$; 20 vs. 180 s: $P < 0.01$). Detailed results are given in Tables 4 and 5 as well as in Figure 4A,B.

To compare the dynamics of recovery between the 2 substances, stimulus intensity was averaged across the concentrations. Then a MANOVA for repeated measures was performed using “odor” and “time” as within-subject factors. Interestingly, there was no significant interaction between the
two factors, indicating that there was no difference between the dynamics of olfactory recovery for H₂S and PEA as expressed by the different functions of the linear slopes.

**Discussion**

Adaptation is a key characteristic of olfactory function, and the underlying mechanisms for olfactory adaptation (and olfactory recovery) are complex. In the present study, we evaluated 2 protocols for assessing olfactory adaptation: 1 for olfactory desensitization and 1 for olfactory recovery.

Twenty healthy, normosmic volunteers were included in the trial. Protocol 1 measured the time to complete subjective desensitization following constant stimulation with either H₂S or PEA in various concentrations. Protocol 2 analyzed intensity ratings of olfactory stimulation with H₂S and PEA in different concentrations after complete desensitization and various periods of recovery.

With protocol 1, we were able to demonstrate that the time to complete desensitization increases with increasing stimulus concentration, and that the concentration of the stimulus has a statistically significant influence on the time to complete desensitization. With protocol 2, the dynamics of olfactory recovery were described, and we could demonstrate that subsequent stimulation was rated as more intense after longer periods of recovery. The odorant itself had no statistically significant effect on the dynamics of recovery.

Standardized protocols for the evaluation of subjective olfactory desensitization or recovery are not available to date. In previous research, olfactory adaptation was regularly tested with the help of detection thresholds (Dalton 1996; Jacob et al. 2003) or perceived intensity levels after short- and long-term exposure to continuous or intermittent stimulation (Dalton 1996; Smith et al. 2010; Kobayashi et al. 2008). In particular, Jacob et al. (2003) examined the ability to detect olfactory stimulation in a series of odor pulses. The degree of adaptation in their trial in terms of detection rates was inversely related to the strength of the stimulus in terms of concentration. This is in accordance with our results from protocol 1, in which the time to complete desensitization was longer when higher concentrations were used. Jacob et al. (2003) could furthermore demonstrate that the detection rates and the dynamics of olfactory adaptation showed significant differences between the substances used and that subjects adapted more rapidly to malodors than to pleasant smells (although they were more sensitive to changes in stimulation with malodors). This finding is also in accordance with our protocol 1, in which H₂S and PEA showed different

<p>| Table 5 Subjective PEA stimulus intensity following olfactory adaptation |
|-----------------------------|-----------------------------|-----------------------------|</p>
<table>
<thead>
<tr>
<th>Concentration (% v/v)</th>
<th>Recovery period (s)</th>
<th>Intensity (VAS 0–10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>5</td>
<td>2.3±2</td>
</tr>
<tr>
<td>10</td>
<td>20</td>
<td>4.2±3.3</td>
</tr>
<tr>
<td>10</td>
<td>80</td>
<td>6.4±3.2</td>
</tr>
<tr>
<td>10</td>
<td>180</td>
<td>6.4±2.8</td>
</tr>
<tr>
<td>20</td>
<td>5</td>
<td>3.8±3.2</td>
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<tr>
<td>20</td>
<td>20</td>
<td>4.9±3.4</td>
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<tr>
<td>20</td>
<td>80</td>
<td>6.1±2.8</td>
</tr>
<tr>
<td>20</td>
<td>180</td>
<td>7.8±2.5</td>
</tr>
</tbody>
</table>

VAS, visual analog scale.

**Figure 4** Logarithmic scale of H₂S (A) and PEA (B) stimulus intensity. Box-whisker plot of the subjective stimulus intensity for the different recovery periods in seconds and concentrations of H₂S in ppm and PEA in percentage. The boxes represent the interquartile range (IQR) with the whiskers extending up to 1.5 times the IQR (meaning the most extreme data points unless they would extend more than 1.5 times the IQR). Outliers outside this range are plotted with a dot. The median is marked as a solid line. This figure is reproduced in color in the online version of the issue.
dynamics in terms of olfactory desensitization as expressed in the differences in the function of the linear slopes (but see also studies indicating that adaptation seems to be more rapid for pleasant stimuli than for unpleasant ones [Croy et al. 2013]). Moreover, our subjects adapted more rapidly (meaning completely desensitized earlier) under stimulation with H$_2$S (malodor) compared with PEA (pleasant smell). Nevertheless, in our study the time to complete desensitization cannot be directly compared between the odorants as the intensity of the odorants was not equal.

Subjective olfactory recovery has been investigated only minimally. In the present study, it is particularly interesting that the dynamics of olfactory recovery after complete desensitization were independent of the concentration used (at least for H$_2$S) and independent of the odorant. Although neural activity cannot be directly linked to perceived intensity, this may be explained by the mechanisms of olfactory adaptation and recovery on the level of the olfactory receptor neuron. One could estimate that the degree of olfactory adaptation in terms of Ca$^{2+}$-dependent negative feedback mechanisms reaches a plateau as soon as complete olfactory desensitization is achieved. After the termination of stimulation, the activity of the adenylyl cyclase and the affinity of the CNG channel need to be restored. Our results suggest that this restoration seems to be relatively uniform and independent of the odorant that has led to desensitization, at least for the 2 substances used in this study. Additional testing with other substances is needed to support this hypothesis.

The study provides 2 robust protocols for psychophysical testing of olfactory adaptation and recovery and enables further research on these 2 basic mechanisms of olfaction.

Conclusion

With the 2 protocols, we were able to measure olfactory desensitization and olfactory recovery in humans. The results confirm current knowledge regarding the dynamics of olfactory adaptation and demonstrate differences in olfactory desensitization between the 2 odorants used. Interestingly, the characteristics of olfactory recovery were independent of the odorant used in this study, indicating that olfactory recovery after complete desensitization may be a uniform process independent of the stimulus used for desensitization.

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