ORIGINAL ARTICLE

Brief Evaluation of Pleasantness of Olfactory and Trigeminal Stimulants

Jan Vodička, MD; Milan Meloun, PhD; Lucie Příhodová, Ing

Objective: To evaluate a brief olfactory test based on pleasantness rating of olfactory and trigeminal stimulants.

Design: Criterion standard.

Setting: University hospital.

Participants: A total of 60 participants (48 healthy and 12 with olfactory loss) aged 16 to 81 years.

Interventions: The new test of odor pleasantness (TOP) based on classification of odorants and trigeminal stimulants into 4 categories was compared with 2 standard tests of subjective olfactometry.

Main Outcome Measure: We evaluated the possible use of the TOP in assessment of olfactory and the trigeminal system in healthy individuals and those with olfactory loss.

Results: All 3 tests demonstrated significant mutual correlation ($P < .01$), and persons with olfactory loss scored lower than healthy participants ($P < .01$). Using exploratory factor analysis and cluster analysis, we found that healthy individuals classified odorants accurately according to the degree of their hedonic character.

Conclusion: The TOP offers outcomes similar to those of standard tests of olfaction that are based on psychophysical testing.

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One of the most important functions of olfaction is to protect the organism against various airborne toxins and against ingestion of spoiled food.[1] The organism perceives most of these substances as malodorous. Conversely, other substances, such as spices and fresh food, invoke delightful sensations. This kind of feeling provides information that the food is edible and that it probably tastes good. Thus, hedonics can be considered as one of the most important functions of olfaction. Herz et al[2] showed that in olfactory perception, the primary response a person has to an odor is the evaluation of how pleasantly or unpleasantly it smells. This conclusion was supported by Bensafi et al,[3] who used psychophysiological methods to study the possible existence of an involuntary affective categorization in olfaction. Their results indicated that unpleasant odors provoked an acceleration of the heart rate during a smelling task (a control condition: a task during which individuals were requested only to inhale odors) as well as during a pleasantness judgment. Their results suggest that individuals involuntarily categorize odors by their pleasantness.[3]

The question is whether an olfactory test assessing odorants for their pleasantness could give us reliable information on a person's ability to smell because significant interindividual variability exists in hedonicity judgments. A rather large number of recently published studies assessed odor pleasantness[2-8]; however, none of the discussed tests of subjective olfactometry are used in routine practice.

Many years ago, Doty[9] proposed a test of odor pleasantness for measuring olfaction. He used amyl acetate as an odorant in different concentrations. Participants were required to rate the perceived intensity and pleasantness of each stimulus. In a later study using 9 olfactory tests, Doty et al[10] found that tests focusing on suprathreshold odor intensity and pleasantness rating may have been measuring sources of variance different than those commonly present in other tests (odor identification, discrimination, detection, memory). Thus, tests focusing on odor intensity and pleasantness may prove to be valuable additions to comprehensive chemosensory test batteries used in clinical settings.

Author Affiliations: Faculty of Health Studies (Dr Vodička) and Department of Analytical Chemistry (Dr Meloun and Ms Příhodová), University of Pardubice, and Department of Otorhinolaryngology–Head and Neck Surgery, Pardubice Regional Hospital (Dr Vodička), Pardubice, Czech Republic.

The most frequently used tests (the University of Pennsylvania Smell Identification Test [UPSIT] and the Sniffin' Sticks test, Identification subtest) (Burghart, Wedel, Germany) are based on selecting the correct odorant name from a list of 4 descriptors. Interpretation of these tests becomes problematic when evaluating children, people with impaired cognitive function, and individuals from diverse cultural and linguistic backgrounds.[11] To overcome difficulties with interpretation existing in the commonly used identification tests, Frank et al[12] introduced the Smell Magnitude Test, which is based on the reduction in sniffing that normally occurs when an odor is encountered. Sniffs of stimuli composed of nothing but nonodorized air are longer and more vigorous compared with sniffs of odorized air. This difference can be used as an indicator of smell function.[12]

Tests of odor hedonics could be useful not only in the clinical practice of ear, nose, and throat specialists but also in neurology and psychiatry. Hudry et al[13] conducted a test to investigate different olfactory tasks in patients with schizophrenia. Pleasantness, familiarity, edibility, and identification, but not intensity, were disturbed in these patients when compared with control participants. Moberg et al[7] revealed a sex-specific disruption in the ability to attribute appropriate hedonic valence to odors in male patients with schizophrenia. Using the Munich Olfaction Test, Rupp et al[8] found that patients with schizophrenia differed from healthy persons in their pleasantness ratings of pure chemicals. Royet et al[4] conducted a study of patients with Alzheimer disease. Differences were observed between the patients and healthy individuals (both old and young) on identification, familiarity, and intensity judgments but not on pleasantness and edibility judgments. In patients with Parkinson disease, major deficits in all cognitive tasks of olfactory judgment (intensity, pleasantness, familiarity, and edibility) were found.[6] Thus, the need for a standard and simple test of odor hedonics seems to be relevant in diagnosis of psychiatric and neurologic diseases.

Furthermore, such a test could be used for differentiation of anosmia and severe hyposmia of various origins. To solve this task, trigeminal stimulants could be potentially useful. It has been proved that patients with olfactory dysfunction have lower trigeminal sensitivity compared with normosmic controls. This seemed to be independent of the cause of the olfactory loss.[14] Even though evaluation of intranasal trigeminal perception is usually based on the lateralization of chemosensory stimuli, trigeminal irritants are usually considered to be pungent and unpleasant by humans.

We developed a simple test that is quickly performed, called the Test of Odor Pleasantness (TOP), which could be used in the general population for clinical purposes. A forced-choice technique is the most common psychophysical testing method used in identification tests.[15,16] Only 1 answer can be selected from 4 descriptors of 1 odorant. We applied the same philosophy to measure pleasantness. Our participants were to decide whether the presented odorant had a pleasant, neutral, unpleasant, or malodorous (very unpleasant) character. We decided to compare the TOP with standard tests (the Odorized Markers Test [OMT] and the Identification subtest of the Sniffin' Sticks test). The goal of our study was to prove the methodology of testing.

METHODS

The study was performed in accordance with the Declaration of Helsinki (Summerset West amendment) concerning guidelines for biomedical research involving human participants. The study was approved by the ethics committee of the Pardubice Regional Hospital, Pardubice, Czech Republic.

PARTICIPANTS

Olfactory function was assessed in 60 individuals; 33 were men and 27 were women. Their mean (SD) age was 39.5 (16.4) years (range, 16-81 years). Healthy people as well as patients with olfactory disorders were tested. We included patients and staff of the Department of Otorhinolaryngology–Head and Neck Surgery of the Pardubice Regional Hospital. Participants reported that their sense of smell was normal in 51 cases, decreased in 5 cases, and completely absent in 4 cases. A total of 48 people did not have any condition that could affect olfaction and considered their sense of smell to be normal. A total of 6 patients had sinonasal disease. Head trauma, upper respiratory tract infection, and toxic etiology were present in 2, 1, and 1 patient, respectively. One patient had undergone previous total laryngectomy, and 1 had undergone radiotherapy of the head and neck region.

STUDY DESIGN

The participants were tested with the OMT, the Sniffin' Sticks test, Identification subtest, and the new TOP. Testing was performed in a quiet room with adequate ventilation. In addition, the person's medical history was taken, and a subjective assessment of smell was recorded (normosmia, hyposmia, or anosmia). For all participants, the medical records in Pardubice Regional Hospital were searched to exclude disease, which could influence olfaction (especially neurological diseases). Endonasal endoscopy was performed in patients with olfactory loss or sinonasal disease. Some of the patients had undergone computed tomographic or magnetic resonance imaging scans to ascertain the etiology of their olfactory loss.

ODORIZED MARKERS TEST

The OMT includes 6 colored and odorized pens (perfumed markers). The black pen smells like liquorice, the yellow one like lemon, the brown one like cinnamon, the blue one like raspberry, the green one like apple, and the red one like strawberry. The OMT is based on spontaneous naming and odor identification from a list of 4 options. First, participants were asked to name the odors they smelled and to identify each marker by a different name. They scored 1 point for naming each of the odors differently. If they were unable to name the odor at all or gave the same name to more than 1 odor, they scored 0 points. Next, the participants were to select 1 correct answer from a list of 4 options; they gained 1 point for every correct identification. The minimum and maximum possible scores were 0 and 12 points, respectively. The creation of this testing technique was described in detail by Vodička et al.[17]

SNIFFIN' STICKS TEST, IDENTIFICATION SUBTEST

A comparison of olfactory function was performed using the Sniffin' Sticks test, Identification subtest, which uses a pen-

like odor-dispensing device. For odor identification, 16 odorants and a list of 4 descriptors were presented to each person. The exact technique of testing was described by Hummel et al.[16]

TEST OF ODOR PLEASANTNESS

Substances and Concentrations

The entire test was developed by the Department of Analytical Chemistry at the Faculty of Chemical Technology, University of Pardubice. Odorants were presented in commercially available felt-tip pens (ART.2739; Centropen a.s., Dačice, Czech Republic). Instead of a liquid dye, the cylinder was filled with odorants (produced by Lachema a.s. [Neratovice, Czech Republic] or by SG spol. s.r.o., Zlín, Czech Republic). In total, 14 pens were filled with 2 mL of various substances (**Table 1**). Based on several experiments performed by the investigators prior to the study, some of the odorants were diluted in water (Table 1). n-Butanol was presented to the participants on 2 occasions; it was presented immediately after water (n-butanol 1) and then again after tetrabuthylmethylether (TBME) (n-butanol 2). The goal was to evaluate the effect of previous odorant on hedonic judgment of the next one.

The odorants were selected based on empirical experience of chemists, who provided an expert opinion on the hedonic tone of diethylamine, mercaptan, and ammonia, which they determined to be fetid or very irritating. n-Butanol, acetic acid, TBME, ethyl acetate, and hexanone were considered to be unpleasant odorants and benzaldehyde a pleasant odorant. Food essence (SG spol. s.r.o.) was added to create pleasant odorants, namely the essence of chocolate, rum, and walnut.

Procedure

An open end of each felt-tip pen was placed 2 cm in front of both nostrils, where it was held for 4 seconds. The participants were asked to categorize the hedonic tone of the odorant into 4 classes: pleasant, neutral, unpleasant, and fetid (or very unpleasant). After approximately 15 seconds, the participant was presented with another odorant.

Scoring

For each odorant, it was determined that its "correct" classification was based on the most frequent response obtained from the healthy participants (**Table 2**). The participant gained 1 point for each correct answer. The total score was calculated as the sum of single points.

STATISTICAL ANALYSIS

The data were analyzed using NCSS2000 (NCSS, Kaysville, Utah) and STATISTICA (StatSoft, Tulsa, Oklahoma) statistical program software. The correlation coefficients of the OMT, the Sniffin' Sticks test (Identification subtest), and the TOP are depicted in **Table 3**. We performed the *t* test to compare data obtained from all 3 tests in healthy participants and in patients with smell deterioration.[18]

The STATISTICA program was used to perform a factor analysis[19] and a cluster analysis.[20] Both analyses were used to determine whether individuals could distinguish between pleasant and unpleasant odorants. In other words, the aim was to determine if the 4 categories used in our technique would be appropriate for separating pleasant, neutral, unpleasant, and very unpleasant substances.

Table 1. Order of Presentation, Names, and Concentrations of the Tested Odorants

Order of the Odorant	Odorant (Manufacturer)[a]	Concentration in Water
1	Cyclohexanone (Lachema)	1:1
2	Chocolate essence (SG)	1:1
3	Water	1:1
4	n-Butanol 1 (Lachema)	1:100
5	Mercaptane (Lachema)	1:10
6	Ethyl acetate (Lachema)	1:50
7	Rum essence (SG)	1:1
8	TBME (Lachema)	1:1
9	n-Butanol 2 (Lachema)	1:100
10	Walnut essence (SG)	1:1
11	Benzaldehyde (Lachema)	1:1
12	Diethylamine (Lachema)	1:50
13	Acetic acid (Lachema)	1:3
14	Ammonia (Lachema)	1:10

Abbreviation: TBME, tetrabuthylmethylether.
[a] Manufacturer information: Lachema a.s., Neratovice, and SG spol. s.r.o., Zlín, Czech Republic.

Table 2. Odorant Categories and the Percentage of Correct Answers in 48 Healthy Participants

Odorant	Category[a]	Correct Answer, %
Cyclohexanon	3	73
Chocolate	1	88
Water	2	67
n-Butanol 1	3	54
Mercaptan	4	69
Ethylacetate	3	96
Rum	1	88
TBME	3	88
n-Butanol 2	2	52
Walnut	1	75
Benzaldehyde	1	63
Diethylamine	3	65
Acetic acid	3	67
Ammonia	4	75

Abbreviation: TBME, tetrabuthylmethylether.
[a] Categories: 1 = pleasant, 2 = neutral, 3 = unpleasant, and 4 = very unpleasant.

RESULTS

Correlation coefficients between all possible pairs of olfactory tests and correlation coefficients between each test and the person's age are presented in Table 3.

All 3 tests demonstrated a significant mutual correlation ($P < .01$) and a negative correlation with age. The mean (SD) scores of all 3 tests are presented in **Table 4**. Compared with healthy participants, persons experiencing loss of olfaction scored lower on all 3 tests ($P < .01$). In healthy individuals, women obtained a higher score than men, but the difference was not statistically significant on any of the tests (for OMT, $P = .09$; Sniffin' Sticks, $P = .09$; TOP, $P = .24$).

Figure 1A shows a 3-dimensional factor loading plot of all 3 factors. The plot focuses on intercorrelation among indicator variables (ie, odorants). Odorants placed near

Table 3. Correlation Coefficients Between All Possible Pairs of Olfactory Tests and Correlation Coefficients Between Each Test and the Participants' Age

Olfactory Test	OMT	Sniffin' Sticks (Identification Subtest)	TOP	Age
OMT	1.00	0.82	0.70	–0.34
Sniffin' Sticks (Identification subtest)	0.82	1.00	0.62	–0.20
TOP	0.70	0.62	1.00	–0.39
Age	–0.34	–0.20	–0.39	1.00

Abbreviations: OMT, odorized markers test; TOP, test of odor pleasantness.

Table 4. OMT, Sniffin' Sticks Test, and TOP Scores for All Participants, Healthy Individuals, and Patients

	Score, Mean (SD)		
Olfactory Test	All Participants (n=60)	Healthy Individuals (n=48)	Patients (n=12)
OMT	8.9 (2.9)	9.8 (1.5)	5.1 (3.7)
Sniffin' Sticks (Identification subtest)	10.8 (2.8)	11.5 (1.9)	7.8 (3.7)
TOP	9.3 (3.0)	10.2 (2.2)	5.8 (3.1)

Abbreviations: OMT, odorized markers test; TOP, test of odor pleasantness.

each other were considered similar (strongly correlated), while odorants placed far were dissimilar (not in correlation).

Figure 1B depicts a comparison of the factor loading plot for the first and the second factors. Pleasant odorants (benzaldehyde, walnut, rum, and chocolate) were distinguished from the group of very unpleasant odorants (mercaptan, ammonia, and diethylamine) and from the rest of the studied odorants.

Figure 2 shows cluster analyses of the studied odorants. Pleasant odorants were distinguished from other odorants. Furthermore, this figure demonstrates that an evaluation of the hedonic character of low concentration of n-butanol (1:100) used in our test correlated with the hedonic character of neutral water. Our intention was to evaluate the effect of preceding odorant on n-butanol. The figure shows that n-butanol, which was presented to the participants on 2 occasions (n-butanol 1, after water; n-butanol 2 after TBME), was considered to be less unpleasant (more neutral) when presented after TBME (unpleasant odorant).

COMMENT

Our TOP proved to possess characteristics similar to those of other olfactory tests used for odor identification. Women outperformed men, and olfactory ability decreased with age.[15,16] The score correlated significantly with scores obtained on tests of odor identification. Patients with various types of impaired olfactory function reached a significantly lower score than the healthy individuals. Using multidimensional analysis, the healthy participants distinguished clearly pleasant odorants (benzaldehyde, walnut, rum, and chocolate) from very unpleasant odorants (mercaptan, ammonia, and diethylamine) and from the rest of the studied odorants.

There are several tests of odor pleasantness used in clinical studies. The test introduced by Doty,[9] mentioned at the beginning of this article, was used in the study by Moberg et al,[7] which focused on patients with schizophrenia. They used 4 different suprathreshold concentrations of amyl acetate diluted in light grade mineral oil (US Pharmaceutical). Participants were asked to rate the pleasantness of the odor on a separate 5-point category scale. Each of the 4 stimulus concentrations were presented 5 times in counterbalanced order. The sum of the intensity and pleasantness rating at each concentration step served as the dependent measures. Currently, odor intensity was measured. In contrast to our test, only 1 odorant was used.

Royet et al[21] introduced measurement of odor pleasantness in a study using 185 odorants. The participants were asked to rate the pleasantness with linear 10-cm rating scale segmented and numbered from 1 to 10. To further indicate the degree of the judgment demanded, the scale extremities were marked "very unpleasant" and "very pleasant." Other qualities were measured, such as intensity, familiarity, and comestibility. From this test,[21] a shorter variant (only 12 odorants) was used to rate pleasantness in Alzheimer disease.[4] Furthermore, this test was used by Hudry et al in studies evaluating pleasantness in patients suffering from schizophrenia[13] and Parkinson disease.[6] Compared with our test, they used rather slightly unpleasant odorants (diethyl ether, acetic acid, and tetrahydrothiophene were diluted 1:10).

In another study using functional magnetic resonance imaging, Royet et al[21] used 126 odorants. The hedonic intensity was rated by using the "finger span" technique,[22] which simulated a visual rating scale by having participants vary the distance between the thumb and forefinger to approximate a linear scale.

Jiang et al[23] used 14 odorants, 7 aromas (almond, cheese, beef, and others), and 7 fragrances (violet, grass, jasmine, lavender, and others). The Self-Assessment Manikin scale, from –4 (as very unpleasant) to 4 (very pleasant), was used to rate the liking of the stimuli. They used this test to evaluate alliesthesia to olfactory stimuli in women either fasting or in a postprandial state.

Ayabe-Kanamura et al[24] measured intensity, pleasantness, familiarity, and edibility of 18 stimuli. Pleasantness was evaluated on 11-point scale with "disgusting" at –5, "neutral" at 0, and "extremely pleasant" at 5. This test was used to study differences in perception of ev-

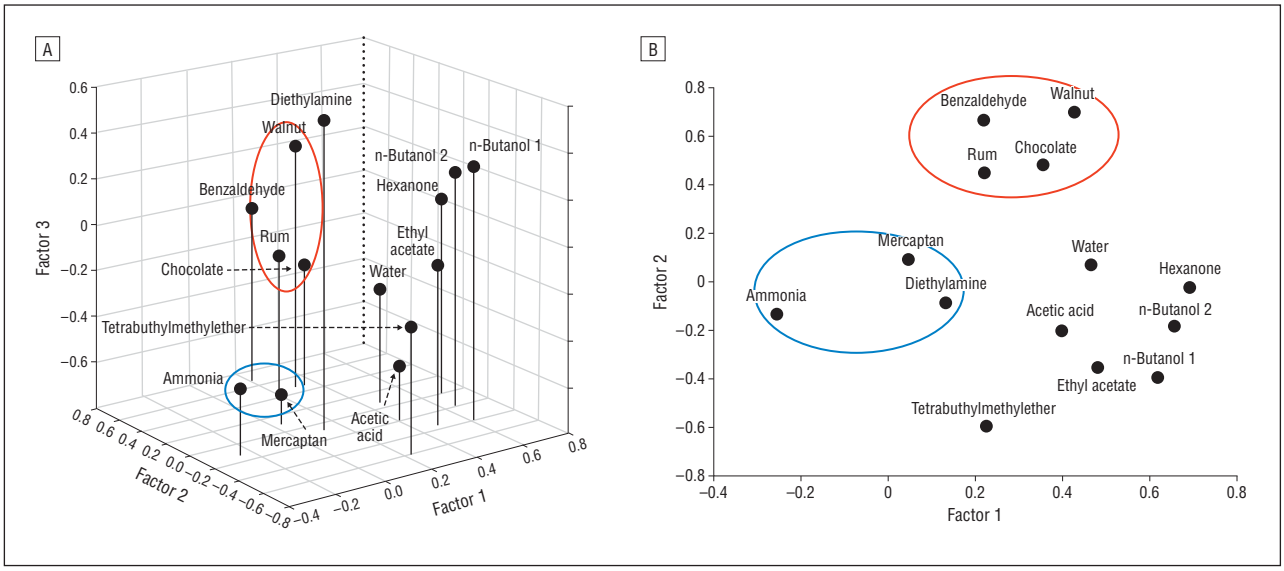

Figure 1. Loading plots. A, Three-dimensional factor loading plot demonstrating the high correlation of pleasant odors (red circle; chocolate, rum, benzaldehyde, and walnut), which are separated from neutral (water) and unpleasant odors (n-butanol 1, which was presented immedicately after water; n-butanol 2, presented after tetrabuthylmethylether [TBME]; hexanone; ethyl acetate; TBME; and acetic acid). Ammonia and mercaptan (blue circle), which are very unpleasant odorants, are separate as well. Diethylamin, which is influenced by the third factor, is placed far from the other odorants. B, Loading plot for the first and the second factors depicting pleasant (red circle), unpleasant, and very unpleasant (blue circle) odorants clearly separated from each other.

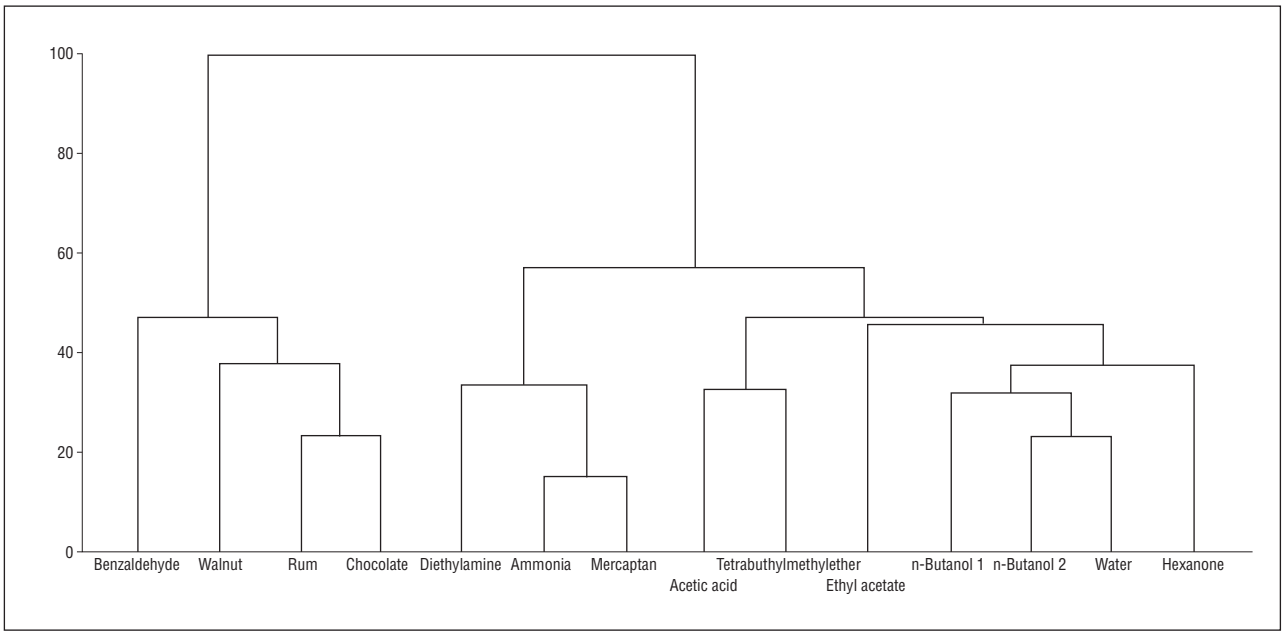

Figure 2. Cluster analyses demonstrate the biggest distance between pleasant odorants and the remaining odorants. Furthermore, very unpleasant odorants are separated from neutral and pleasant odorants. n-Butanol was presented twice. The second presentation of n-butanol (n-butanol 2) is placed closer to water then the first presentation (n-butanol 1).

eryday odors in Germany and Japan. The same scale of measuring pleasantness was used by Herz et al[2] and Distel and Hudson.[5] Rupp et al[8] used this scale to measure various olfactory deficits in male patients with schizophrenia. In contrast to our study, they used everyday odorants with slight hedonic character (eg, soy sauce, Japanese tea, chocolate, almond, anise, and others).

In all the tests, evaluation of pleasantness was scored on a symmetrical linear scale, from unpleasant to pleasant ones. In our test, participants were forced to choose 1 of the 4 categories for odor pleasantness.

The advantage of our test is a fast hedonic evaluation of odorants owing to easy scaling and using strong stimulants. Moreover, strong trigeminal stimulants are used, which could possibly detect patients with serious olfactory deficit.

We decided to use 2 categories for unpleasant odorants owing to their importance in protection of organisms.[1] Higher autonomic arousal was evidenced in response to presentation of an unpleasant odorant compared with a pleasant one.[25] Moreover, the reaction time and velocity of sniffing is influenced by malodorants more

than by pleasant odors. The reaction time to malodorants was measured by Jacob and Wang,[26] who found that valeric acid, a malodorant, was detected more rapidly than amyl acetate, a pleasant odorant. A similar result was obtained by Bensafi et al,[3] who concluded that unpleasant odors were detected more rapidly than neutral or pleasant odors.

Based on changes in the velocity of sniffing on presentation of pleasant and unpleasant odorants to their participants, Frank et al[12] introduced the Sniff Magnitude Test. They selected 2 malodorants—and not a single pleasant-smelling odor—to compare their test with the UPSIT and to evaluate its usefulness as a clinical measure of olfactory function.[27] Furthermore, it was established that malodors exhibited stronger correlations than nonmalodors and elicited greater sniff suppression.[28]

Konstantinidis et al[29] studied age effects in overall odor identification performance and found that age-related deficits were odorant specific. In particular, odors perceived as unpleasant showed age invariance, whereas odors rated as pleasant exhibited age sensitivity. Because identification of unpleasant odors was better preserved compared with identification of pleasant odors, this finding could allude to the importance of malodorant identification in people's lives.

We also decided to use trigeminal stimulants because strong hedonic ratings can be observed when trigeminal irritants, such as acetic acid and ammonia, are presented. Also, tight functional connection of the trigeminal to the olfactory system was proved. On an electrophysiological basis, Hummel et al[30] measured chemosomatosensory event-related potentials in response to suprathreshold trigeminal stimuli; they found a decrease of trigeminally mediated sensations in patients with smell deterioration compared with healthy persons. Their recommendation was to include an evaluation of trigeminal responses as an integral part of diagnostic testing for smell disorders.

It is well documented that hedonic characteristics of odors are, to a certain extent, learned and that they are affected by events experienced in other modalities.[31] Our work demonstrates that in an ethnically homogeneous group, stable responses were obtained on presentation of odorants possessing different hedonic characters. Generally, there are reports of good correlations of odorants' hedonic. For instance, when creating the Sniffin' Sticks test, Hummel et al[16] selected pleasant smells to make it more appealing to their study participants. Their ratings of the odorants' hedonic tone exhibited a good correlation with data obtained by Dravnieks et al.[32]

Our study strived to include extremely pleasant and unpleasant odors to gain as high interindividual stability as possible. According to Cain and Johnson,[33] their participants exhibited the same agreement concerning hedonically neutral odors as well as concerning extremely pleasant and unpleasant odors. Conversely, in a study published by Alaoui-Ismaïli et al,[25] weak correlation was obtained between interparticipants' hedonic evaluations despite the fact that odorants with strong negative hedonic value were used (acetic acid and butyric acid). After inhaling the odorants, individuals were requested to identify the odorants and mark them on an 11-point hedonic scale. In our study, an identification of odorants was not performed, and only a 4-point hedonic scale was used. Therefore, our participants focused only on judging pleasantness, and they were offered only 4 options regarding the odorants' hedonic tone.

In contrast, Khan et al[34] focused on predicting the odor of molecules based on their physicochemical features. They found that the primary axis of perception was odor pleasantness and that the primary axis of physicochemical properties reflected the primary-axis of olfactory perception. They were able to predict the pleasantness of novel molecules by their physicochemical properties alone. Their findings suggest that olfactory pleasantness is also partially innate. Their conclusion supports the use of pleasantness ratings in clinical olfactory tests; however, an international study using the TOP is needed to confirm this idea. Furthermore, the selection of test odorants is a subject that demands further investigation.

We present the first preliminary data of the olfactory test based on brief evaluation of odor pleasantness. Although the results are promising, the study has certain limitations that need to be taken into account. First, only 2 psychophysical tests of olfaction were used to compare the results. Both of these tests were based on suprathreshold testing (identification); one (the OMT) is considered solely as a screening test, and normative data for the Czech population for the other one (Sniffin' Sticks test, Identification subtest) are not available at this time. Second, no test was used to determine olfactory threshold. Thus, it was not possible to precisely establish the degree of an olfactory loss. Retesting was not performed to confirm or exclude the reliability of the TOP. However, a test-retest study concerning suprathreshold pleasantness rating was described by Doty et al.[35] The correlation coefficient reached 0.78, which was one of the highest among the tests used in their study. Although trigeminal stimulants were represented in the TOP, no specific test of intranasal function of the fifth cranial nerve was used (eg, the lateralization test introduced by Wysocki et al[36]). All of these shortcomings need to be evaluated in future studies.

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Correspondence: Jan Vodička, MD, Klinika otorinolaryngologie a chirurgie hlavy a krku, Kyjevská 44, 532 03 Pardubice, Czech Republic (jan_vodicka@hotmail.com).
Author Contributions: Drs Vodička and Meloun had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. *Study concept and design:* Vodička. *Acquisition of data:* Vodička and Příhodová. *Analysis and interpretation of data:* Vodička, Meloun, and Příhodová. *Drafting of the manuscript:* Vodička. *Critical revision of the manuscript for important intellectual content:* Meloun and Příhodová. *Statistical analysis:* Meloun and Příhodová. *Obtained funding:* Vodička. *Administrative, technical, and material support:* Meloun. *Study supervision:* Vodička.

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