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Measurement of Sensitivity to Olfactory Flavor: Application in a Study of Aging and Dentures

Valerie B. Duffy, William S. Cain[1] and Ann M. Ferris[2]

School of Allied Health, University of Connecticut, Storrs, CT 06269-2101, [1]Chemosensory Perception Laboratory, Department of Surgery (Otolaryngology), University of California, San Diego, La Jolla, CA 92093-0957 and [2]Department of Nutritional Sciences, College of Agriculture and Natural Resources, University of Connecticut, Storrs, CT 06269-4017, USA

Correspondence to be sent to: Dr Valerie B. Duffy, School of Allied Health, University of Connecticut, 358 Mansfield Road, Box U-101, Storrs, CT 06269-2101, USA. e-mail: vduffy@uconnvm.uconn.edu

Abstract

Olfaction involves a dual sensory process for perceiving odors orthonasally (through the nostrils) and retronasally (through the mouth). This investigation entailed developing a measure of sensitivity to an odor delivered in an orally sampled food (orange flavoring in a sucrose-sweetened gelatin) and examining sensitivity in the elderly. In experiment 1, olfactory flavor sensitivity was 49 times lower in elderly ($n = 21$) than in young ($n = 28$) subjects. In experiment 2, with 73 elderly women, higher olfactory flavor sensitivity correlated significantly with higher orthonasal perception (Connecticut Chemosensory Clinical Research Center test). Some women, however, exhibited low olfactory flavor sensitivity despite high orthonasal perception; none had high olfactory flavor sensitivity and low orthonasal perception. Those who wore complete or palatal covering dentures had lower olfactory flavor sensitivity than those who were dentate or wore dentures that did not cover the palate. Through multiple regression analysis, orthonasal perception and denture status were found to be independent contributors to predicting olfactory flavor sensitivity. In summary, elderly subjects showed depressed olfactory flavor sensitivity (i.e. retronasal sensitivity) that related to poor orthonasal olfactory perception and denture characteristic. Thus, while good orthonasal olfaction may be necessary for good olfactory flavor sensitivity, it is not sufficient. Other factors, some associated with oral conditions, may impede release and retronasal transport of odors from the mouth to the olfactory receptors.

Introduction

There are two pathways to olfactory perception: odors reach the olfactory epithelium through the nostrils (orthonasal olfaction) and through the nasopharynx (retronasal olfaction). Retronasally perceived odorants are generally delivered in a liquid phase during eating or drinking and, when combined with gustatory, somatosensory and auditory sensation, meld into a composite sensation of flavor. Perceiving odorants orthonasally and retronasally have been thought to be qualitatively different experiences (Rozin, 1982). However, odorants delivered retronasally in the vapor phase (thus removing the interference from gustatory and somatosensory sensations) can produce similar experiences to odorants delivered orthonasally (Pierce and Halpern, 1996). The intent of the present studies is to examine olfactory perception as intraoral liquid stimulation as during eating or drinking. For the purpose of this paper, we use the term 'olfactory flavor' to refer to the olfactory component of this stimulation.

Most olfactory studies are designed to measure only orthonasal perception. These studies reveal a level of olfactory impairment that is greater in the elderly than in young adults [see the following reviews (Cain and Stevens, 1989; Weiffenbach, 1991; Schiffman, 1992, 1997; Murphy, 1997)]. Fewer studies test olfaction retronasally through liquid flavorings placed inside the oral cavity; these also find diminished perception in older adults [e.g. (Murphy, 1985; Stevens and Lawless, 1981; Stevens and Cain, 1986; Cain *et al.*, 1990; Schiffman and Warwick, 1993)]. Both orthonasal and retronasal olfactory perception could be impaired by defects in the olfactory system, whether due to aging alone or in combination with pathology and environmental insults. The retronasal olfactory experience is more sophisticated than the orthonasal experience, as it can require adequate mastication to release olfactory volatiles, which are pumped retronasally to the olfactory region by sufficient mouth and swallowing movements (Burdach and Doty, 1987; Doty, 1989).

Investigators have documented age-related olfactory impairment through a variety of psychophysical measures, including odor detection, quality discrimination, identification, recognition, estimation of magnitude and estimation of similarity (Schemper *et al.*, 1981; Doty *et al.*, 1984;

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Stevens and Cain, 1985; Eskenazi *et al.*, 1986; Cain and Gent, 1991; de Wijk and Cain, 1994a,b). While compromise of olfactory perception in the elderly is evident in virtually all these measures, odor detection, as reflected in absolute threshold, provides the most fundamental and interpretable index of deficit (Stevens and Dadarwala, 1993). Even if threshold does not directly measure ability to perceive concentrated stimuli, it does set a framework to evaluate changes in suprathreshold functioning. In olfactory perception studies, a substantial elevation in threshold accompanies diminished perceptual abilities throughout the suprathreshold range [see (Stevens and Cain, 1987) for a review]. Thus, if the odor threshold in older adults is substantially above that of young adults, then perceived intensity of concentrated odorants would be diminished as well.

Measured odor thresholds in older adults have ranged from ~2-fold to 100-fold above those in young adults (Cain and Stevens, 1989; Wysocki and Gilbert, 1989; Stevens and Cain, 1993; Stevens and Dadarwala, 1993). This range in difference between old and young could genuinely represent age-related changes in olfactory perception and/or could be the result of differences in design of the studies. These studies have varied in methodology (number of subjects, psychophysical technique, mode of stimulus delivery, odorant) as well as in the characteristics of the subjects (age, ethnicity, general health, dental health, geographic residence, place of work). All of these factors may influence the stability of the olfactory threshold [see (Doty, 1997) for a review], especially for the elderly (Stevens and Dadarwala, 1993). Some of these variables were held constant in studies of threshold to 1-butanol with subjects from New Haven, CT. From these studies, threshold differences between young and elderly ranged from 30-fold to 100-fold (Cain *et al.*, 1990, 1995; Cain and Gent, 1991; Patterson *et al.*, 1993; Stevens and Dadarwala, 1993). The small variation of ~3-fold among these studies accounts for only ~7% of the total range seen in the literature, which makes it clear that much concerning determinants of sensitivity remains to be understood (Rabin and Cain, 1986).

A question of interest concerns whether thresholds for olfactory flavor fall within the range found for odors. Delivered in the vapor phase, retronasal olfactory thresholds can exceed orthonasal thresholds (Voirol and Daget, 1986). The elderly show poor performance in identification of the presence or absence of olfactory flavorings in foods (Schiffman, 1977; Murphy, 1985; Cain *et al.*, 1990); thus, elevation of olfactory flavor threshold appears likely. This study proposes a way to measure olfactory flavor thresholds to examine both differences between young and elderly subjects and predictors of threshold level in elderly subjects. The olfactory flavor threshold test was designed to mimic perceptual experiences of food and be suitable for laboratory- or field-testing. In a simple task, subjects orally palpated a sweetened gelatin with an orange flavoring that varied from subthreshold to threshold concentrations. The first of two studies verified that aging subjects perform substantially below young subjects; the second employed the task in a field study of healthy elderly women to examine predictors of olfactory flavor sensitivity (a clinical measure of orthonasal perception, age, and denture status).

Experiment 1: sensitivity to flavor in young and elderly subjects

Materials and methods

Subjects

Twenty-one young subjects (11 females, 10 males) from 18 to 32 years of age (average 24 ± 4 SD) and 28 elderly subjects (22 females, 6 males) from 63 to 93 years of age (average 74 ± 7 SD) participated. The subjects were recruited in New Haven, the young with posters and the elderly through presentations at senior centers. All subjects professed average or better health for their age. Young subjects were tested in the laboratory and the elderly at their senior centers. The investigation was approved by the University of Connecticut Human Subjects Committee; subjects gave informed consent and received compensation for participation.

Materials

The stimulus consisted of a full-strength, water-soluble, blended natural and artificial orange flavoring (Product #106290, Cultor Food Science, Ardsely, NY), which was dissolved in a sweetened gelatin base. This flavoring is used commercially to flavor beverages. The stock solution, denoted dilution step 1, contained 120 ml of orange flavoring, one cup of sucrose and four packages of Knox® gelatin dissolved in six cups of deionized water. Subsequent dilutions halved the concentration of flavoring 11 times to produce a series of 12 concentrations (weakest member: step 12). Pilot testing showed that step 12, a dilution of 2048-fold from stock, lay safely under the threshold for most young subjects and step 1 lay above the threshold for most elderly subjects. By design, the sweet background, 0.44 M sucrose, had a sweetness similar to commercial gelatin desserts. The combination of orange and sweet gave the product a more natural character than its unsweetened version would have.

Portions of stimulus were cubes (~10–15 cm^3) presented in medicine cups with sampling spoons. The cubes were chilled to 4.4–7.2°C so that they would minimize olfactory stimulation until palpated in the mouth. The cubes did not present a masticatory challenge, yet felt like food. The addition of the olfactory flavoring did not modify the visual characteristics of the gelatin sample. In order to establish whether discrimination depended on sensory cues other than those from olfaction, two anosmic subjects participated in the entire threshold procedure (see below). These subjects were not able to obtain four correct answers in the discrimination between blanks and flavor stimuli, even at the highest concentration of flavoring.

Procedure

A two-alternative forced-choice (2AFC) version of the ascending method of limits, common in chemosensory testing, served to measure threshold (Cain *et al.*, 1988). At testing, the experimenter read the following instructions to the subject: 'I will present you with two gelatin samples. Both are made with gelatin, table sugar and water. However, one has an orange flavor in addition to the sweet taste. I would like you to tell me which sample has the orange flavor.' The subject took the samples by spoon from medicine cups. The subject rinsed with water before sampling again and expectorated the rinse. Testing began with the lowest concentration and progressed in steps to higher levels upon incorrect choices. Concentration remained the same after correct choices. A minute elapsed between trials. Testing ceased when the subject made four successive correct choices, and this concentration was designated as the threshold. Replicate sessions occurred about 1 week later.

Results

Compared with the young, elderly subjects needed 49 times the concentration of olfactory flavoring for consistent detection. Average threshold, expressed as dilution step, equaled 2.7 ± 2.4 SD (range 0–7.5) for elderly subjects and 8.3 ± 2.3 (range 3.0–12.0) for young subjects. Means may actually underestimate the difference because some elderly subjects failed to detect the maximum available concentration and were therefore assigned a threshold one step above the maximum. When assessed for the combined sample of young and elderly subjects, test–retest reliability (r) equaled 0.88 ($P < 0.0001$). When assessed separately, reliability equaled 0.80 ($P < 0.0001$) for the elderly group and 0.63 ($P = 0.005$) for the young group. There were no significant sex differences in threshold in either age group (Kolmogorav–Smirnov test, $P \geq 0.1$).

Discussion

The task discriminated olfactory flavor thresholds of elderly and young with high resolution, to almost two and one-half standard deviations (i.e. $z = 2.5$). The use of chilled gelatin allowed samples to pass into the mouth with minimal orthonasal olfactory cues and eliminated the need for encumbrances, such as nose clips. The task would therefore seem reasonable for demonstrating the amount of olfactory flavor a person might extract from food under somewhat unchallenging circumstances of eating. Eating can present an array of challenges to the extraction of flavor, from assessment of olfactory subtleties too fine for instruments to detect to gross distinctions between edible and inedible substances. Perhaps no task or index will capture all of these ranges, but our method appears to be on task for the measurement of threshold.

Experiment 2: flavor threshold, olfactory competence and dentures

Further consideration of the olfactory flavor threshold task prompts the question, does it convey information about olfactory perception that is different from that obtained by a measure of orthonasal functioning? If the olfactory flavor threshold test is a reasonable assessment of olfactory perception, an individual who performs poorly on an orthonasal measure should perform poorly on the olfactory flavor threshold task. However, the olfactory flavor threshold might provide additional information that is relevant to olfactory experiences that accompany eating.

A clinical test of olfactory perception, such as the Connecticut Chemosensory Clinical Research Center (CCCRC) test, offers one benchmark of orthonasal functioning (Cain *et al.*, 1988; Cain and Rabin, 1989). Persons who perform below a criterion level on this test—which combines a threshold with a task of identification—are deemed impaired, not only because of their relative infrequency in the population, but also because those who perform below that level often complain to physicians about loss. Elderly persons perform less well on the CCCRC test than the young and more often score below the criterion for impairment set for nonelderly persons. It appears, however, that few elderly approach physicians about their loss, either because they fail to notice the slow accumulation of deficit or because they accept it as normal (Stevens, 1989). Since incremental loss of smell begins in middle age, net loss in old age may readily escape notice (Eskenazi *et al.*, 1986; Cain *et al.*, 1990, 1995; de Wijk and Cain, 1994a, 1994b). Persons who seek medical attention for olfactory loss tend to report rapid changes.

Also of interest was the question of whether conditions in the mouth influenced olfactory flavor perception. In experiment 1, many elderly subjects wore dentures (20 of 28). Thus, we may have revealed a difference in olfactory flavor threshold between elderly with dentures and young subjects. Dentures may handicap masticatory functioning and mouth movements and, as a result, alter perception of olfactory food flavor (Zarb, 1997). In experiment 2, we recruited elderly subjects who had dentures and those who were dentate, and defined the 'dentures group' as those who wore complete maxillary dentures that covered the palate. The interactions between the palate and the tongue appear critical during both the voluntary and involuntary stages of swallowing to create the changes in the intraoral pressure sufficient to pump volatiles retronasally to the olfactory receptors (Burdach and Doty, 1987). We hypothesized that the average olfactory flavor threshold would be higher in those with palatal covering dentures than in those who were dentate or had minimal partial dentures. The independent and combined influences of denture status, orthonasal olfactory functioning (as measured by the CCCRC test) and age on olfactory flavor threshold were examined through multiple regression analysis.

Materials and methods

Subjects

Seventy-three elderly women from 65 to 93 years of age (average 76 ± 6) were recruited from senior housing complexes in New Haven, only 10 of whom had participated in experiment 1. The women were independent-living in separate apartments and were primarily Caucasian. For study participation, each had to meet and additional criteria for functional status. The level of functional status was quantified with the Older Americans Resources and Services Multi-Dimensional Functional Assessment Questionnaire (OMFAQ) (Fillenbaum, 1988), which provides a cumulative index, ranging from 'excellent functioning' to 'totally impaired', based on social support status, economic status, physical health, mental health (including cognitive ability) and self-care capacity. To meet study criteria, all women needed to pass the cognitive screen of the OMFAQ (i.e. Short Portable Mental Status Questionnaire) and score no worse than 'mildly impaired' on the cumulative index. The first author was certified to administer and score this questionnaire by the Center for the Study of Aging and Human Development, Duke University.

The data collection was completed in the subject's home over four sessions and started with the OMFAQ. If the subject met the above criteria, they were then given either the CCCRC or olfactory flavor threshold tests. For the olfactory testing, we aimed to have replicate CCCRC testing and olfactory flavor testing, one test per week, over 4 weeks, with the order of testing counterbalanced. Fifty-seven women participated in repeat CCCRC testing and 68 in repeat olfactory flavor threshold.

Procedure

The procedure used to assess detection of orange flavor followed that of experiment 1. The CCCRC test entailed measurement of threshold to 1-butanol (2AFC, ascending method), followed by identification of seven odorants (baby powder, chocolate, cinnamon, coffee, mothballs, peanut butter and Ivory® soap) and reaction to a trigeminal probe (Vicks Vapo-Steam®). Butanol vapor was delivered from the headspace of 270 ml polypropylene squeeze-bottles with pop-up spouts that allowed testing of each nostril separately (Cain *et al.*, 1988). Successive 3-fold aqueous dilutions from a concentration of 4% (step 0) produced a series of 11 members, with step 10 the weakest concentration. In such a series, vapor-phase concentration varies from 3055 ppm to 46 ppb. The experimenter squeezed the polypropylene bottles for the subjects to assure a consistent release of odorant from the bottle. Identification was also tested one nostril at a time.

The maximum score a person may receive on the CCCRC is seven and the minimum zero. A score of seven requires high performance on both threshold and identification for each nostril. Scoring is as follows: when a person detects butanol at dilution step 7 or better, she obtains a maximum subscore of seven; when she identifies all seven odorants, she receives a second subscore of seven. The average of these two subscores equals the net score for a given nostril. The average across the two nostrils gives a final score. If a subject yields a threshold lower than step 7, she receives a subscore equal to the dilution step at the threshold, step 6 through step 0. If the subject identifies fewer than seven odorants, she receives a score for the number she did identify. For persons under 65 years of age, a composite score of six or better constitutes normosmia by the norms of the test. For persons 65 or above, a score of five or better constitutes normosmia (Cain and Rabin, 1989).

Analyses

For analysis of the role of dentures, the sample of 73 was divided into 27 (average age 76 years) who were either dentate or wore dentures that left the palate uncovered and 46 (average age also 76 years) who were edentulous with dentures that covered the palate. A standard multiple regression was performed between olfactory flavor threshold as the dependent variable and CCCRC score, denture group and age as independent variables. The CCCRC score was transformed based on evaluation of the assumptions of multiple regression (Tabachnick and Fidell, 1989). This reduced the outliers and improved the normality, linearity and homoscedasticity of the residuals.

Results

Olfactory flavor threshold averaged almost the same in the group of 73 (2.6 ± 1.9) as in the previous, smaller group of elderly subjects (2.7 ± 2.4). Test–retest reliability equaled 0.61 (P = 0.0001), whereas it had equaled 0.80 in the previous group of elderly subjects. The mean CCCRC score equaled 4.8 ± 1.7. The CCCRC scores were skewed toward higher functioning, but nevertheless contained a substantial number below normosmia: five subjects were anosmic (scores 0–2), 26 severely to mildly hyposmic (scores 2–5) and 42 normosmic (scores above 5) (see Table 1). Test–retest reliability for the CCCRC test equaled 0.84, 0.77 for butanol score and 0.83 for identification score (Cain and Rabin, 1989).

Olfactory flavor sensitivity and CCCRC score correlated significantly though modestly (0.35, $P < 0.005$). The form of the association between CCCRC score and olfactory flavor threshold revealed that high performance on the flavor test corresponded to relatively high performance on the CCCRC test, but high performance on the CCCRC test did not necessarily correspond to high performance on the olfactory flavor test (see Table 1). The competence implied by a high CCCRC score seems important, but not sufficient, to score well on the olfactory flavor threshold. The group with dentures that covered the palate had a significantly higher threshold than those who were dentate or who wore dentures that did not cover the palate, 3.3 ± 2.2 versus 2.2 ± 1.7 [$t(71) = 2.48$, $P < 0.05$], a factor of 2.1 (Figure 1). In

Table 1 Joint frequencies of scores on the CCCRC test (scale = 0–7; 0 = anosmia and >6 = normosmia) and flavor task (dilution step from 0 to 10; 0 = highest threshold) for 73 elderly women (above 65 years old)

Flavor	0–0.9	1–1.9	2–2.9	3–3.9	4–4.9	5–5.9	6–7
0–0.9	3		2	2	2	2	4
1–1.9	1	1	1	2	4	2	4
2–2.9			1	3	4	1	2
3–3.9			1	2		4	2
4–4.9					1	5	6
5–5.9						1	2
6–7					1	4	3

regression analysis, the multiple R equaled 0.44 and was significantly different from zero, $F(3,69) = 5.634$, $P < 0.005$. Both CCCRC score (0.33, $P < 0.005$) and denture-status (0.26, $P < 0.05$) contributed significantly to prediction of olfactory flavor threshold score, though age did not ($r = 0.08$, $P = 0.49$).

Discussion

Our olfactory flavor threshold test appears to offer an adequate test of sensitivity to an olfactory flavoring with the following results:

1. Anosmics exhibited no ability to discriminate samples with flavoring (in this case orange) from those without flavoring, which indicates that this detection task apparently taps only the olfactory component of flavor.
2. The olfactory flavor threshold test exhibited respectable test–retest reliability, both across the two age groups ($r = 0.88$) and within the elderly themselves ($r = 0.80$ and $r = 0.61$). The lower value obtained in the second experiment suggests virtue in making the measurements twice, preferably with an interval between, and averaging the two, as was done in these experiments. Although there are limitations with the reliability of single measurements, two should reduce unexplained variance to just 15% [i.e. $(1 - r)^2 = (1.00–0.61)^2 = 0.15$ for two measurements] in test–retest reliability. The task takes only ~20–30 min; two measurements consume only about 1 h of a subject's time.
3. The olfactory flavor threshold test differentiated young adults from elderly adults with a sensitivity that rivals or exceeds comparable tests, such as odor thresholds (Stevens and Dadarwala, 1993).
4. The olfactory flavor threshold test shows an association with olfactory competence as measured through the nostrils with the CCCRC. However, some elderly women had an elevated olfactory flavor threshold despite more normal olfactory functioning.
5. Dentures that covered the palate were associated with an elevated olfactory flavor threshold; this effect was independent of olfactory functioning as measured with the CCCRC test. Dentures could diminish olfactory flavor perception by interfering with chewing and mouth movements. Although the stimulus did not offer a masticatory challenge, masticatory movements, as well as swallowing movements, may be necessary to create sufficient pressure to pump the olfactory volatiles retronasally to the olfactory bulb (Burdach and Doty, 1987). The study did not control for the amount and degree of mouth movements as subjects sampled the gelatin. Difference in flavor threshold between young and elderly subjects and between elderly with and without palatal-covered dentures could have resulted from differ-ence in ability to release olfactory volatiles from the gelatin. The relationship between masticatory ability measured directly or indirectly by functional characteristics of the dentures (e.g. stability, retention, occlusion) deserves further attention. The palatal-covered dentures could have made it difficult for the individual to clear the stimulus from the mouth because either the stimulus adsorbed to the denture material or was retained between the palate and the denture. A reduction in olfactory flavor sensitivity may also not be specific to dentures. Additional oral conditions may also impair olfactory flavor perception. It is uncertain if some of the complaints of diminished food enjoyment that accompany problems with oral health, such as soreness in the mouth and periodontal diseases (Lipton *et al.*, 1993; Ship, 1993; Ship *et al.*, 1995; Griep *et al.*, 1996), are related to loss of olfactory food flavor. Future studies should provide more conclusive data on the influence of oral conditions on olfactory flavor perception.

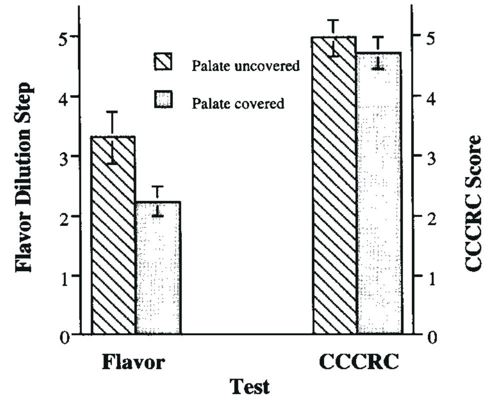

Figure 1 Elderly women who wore dentures that covered the palate ($n = 46$) performed significantly worse on the flavor task [$t(71) = 2.48$, $P < 0.02$], but not on the CCCRC test, than women who were dentate or who wore partial dentures that did not cover the palate ($n = 27$).

The olfactory flavor threshold test offers a relatively simple measure of olfactory flavor perception within a model food. Gelatin is a food medium that permits the manipulation of flavor and thus can be used to test suprathreshold perception of olfactory flavorings (Dabrila and Duffy, 1996) and various levels of sweetness, saltiness, etc. The combination of stimulation from chemosensory stimuli both within and across modalities may offer opportunities to see net effects not evident with single stimulus or single modality (Stevens *et al.*, 1991; Stevens and Cain, 1993).

The findings of this study have implications for understanding olfactory perception in the elderly and studying the association between olfactory perception and food and nutritional parameters. The elderly may show greater impairment to retronasal perception of olfactory flavor than to orthonasal perception of odors as the result of oral conditions which influence chewing and mouth movements. The interplay between olfactory perception and food behaviors can only be fully explored by using measures of chemosensory function that pertain to the dynamic aspects of eating (de Graaf *et al.*, 1994). The olfactory flavor threshold test reported in this paper may provide additional information about the association between olfactory dysfunction and nutritional risk in the elderly (Duffy *et al.*, 1995).

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