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Sensory Characterization of the Irritant Properties of Oleocanthal, a Natural Anti-Inflammatory Agent in Extra Virgin Olive Oils

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Abstract

Oleocanthal is an olive oil phenolic possessing anti-inflammatory activity. Anecdotal evidence suggests that oleocanthal elicits a stinging sensation felt only at the back of the throat (oropharynx). Due to this compound possessing potentially health-benefiting properties, investigation into the sensory aspects of oleocanthal is warranted to aid in future research. The important link between the perceptual aspects of oleocanthal and health benefits is the notion that variation in sensitivity to oleocanthal irritation may relate to potential differences in sensitivity to the pharmacologic action of this compound. The current study assessed the unique irritant attributes of oleocanthal including its location of irritation, temporal profile, and individual differences in the perceived irritation. We show that the irritation elicited by oleocanthal was localized to the oropharynx ($P < 0.001$) with little or no irritation in the anterior oral cavity. Peak irritation was perceived 15 s postexposure and lasted over 180 s. Oleocanthal irritation was more variable among individuals compared with the irritation elicited by CO_2 and the sweetness of sucrose. There was no correlation between intensity ratings of oleocanthal and CO_2 and oleocanthal and sucrose ($r = -0.15$, $n = 50$, $P = 0.92$ and $r = 0.17$, $n = 84$, $P = 0.12$, respectively), suggesting that independent mechanisms underlie the irritation of CO_2 and oleocanthal. The unusual spatial localization and independence of acid (CO_2) sensations suggest that distinct nociceptors for oleocanthal are located in the oropharyngeal region of the oral cavity.

Key words: individual differences, irritation, oleocanthal, somatosensory system

Introduction

Newly pressed extra virgin olive oils (EVOOs) contain the olive oil phenolic, (–)-decarboxymethyl ligstroside aglycone, also known as oleocanthal (oleo- for olive, canth- for sting, and al- for aldehyde). Anecdotal evidence suggests that upon consumption of these EVOOs, oleocanthal elicits a concentration-dependent irritation at the back of the throat (oropharynx) (Beauchamp et al. 2005). Oleocanthal has been shown to mimic the pharmacology of ibuprofen, also an oropharyngeal irritant with similar structure to oleocanthal (both contain benzene rings and are branched), in that oleocanthal has the capacity to inhibit the same cyclooxygenase enzymes in the inflammatory pathway as does ibuprofen, making oleocanthal a natural non-steroidal antiinflammatory drug (Breslin et al. 2001; Beauchamp et al. 2005). The potential relationship between health benefits and the Mediterranean diet makes oleocanthal a compound of interest, and an investigation into the apparent unique sensory aspects of oleocanthal is warranted to help direct future research. The important link between the perceptual aspects of oleocanthal and health benefits is the notion that variation in sensitivity to oleocanthal irritation may relate to potential differences in sensitivity to the pharmacologic action of this compound.

Mucous membranes in the oral, nasal, and pharyngeal regions are particularly sensitive to the effects of specific irritant chemicals. To stimulate the nociceptive and thermal neurons, chemicals must travel through the epithelia. Mucous membranes have shallow innervation, making them particularly sensitive to chemical stimuli. The need for the chemicals to penetrate the membrane in order to stimulate the nociceptors and thermal receptors is likely the reason that chemesthetic sensations typically take longer than tastes and smells to develop and decline (Green 1996; Walker and Prescott 2003; Cain et al. 2006).

Beauchamp et al. (2005) used a sensory-directed approach to isolate and identify oleocanthal. Although this approach

has proved useful in the identification of oleocanthal, reproducibility of human subject ratings and individual variability in sensitivity to oleocanthal remains unexplored. Furthermore, the location of irritation has not been formally tested. Therefore, the aim of the current study was to further characterize the perceptual attributes of oleocanthal. This included the investigation of spatial and temporal patterning of irritation together with individual differences in perception of oleocanthal irritation. This information will help to elucidate the psychophysical properties of an unusual irritant and the very popular food ingredient that elicits it.

General materials and methods

Experiments were conducted at 2 independent sensory testing centers, in Melbourne, Australia, and Philadelphia, PA. The concentration of oleocanthal used in the studies varied due to natural variances in phenolic composition in EVOOs. Oleocanthal levels were quantified by high-performance liquid chromatography (HPLC). For the work conducted in Philadelphia, PA, the method by Impellizzeri and Lin (2006) was used. For work conducted in Melbourne, Australia, a modified method of that of Impellizzeri and Lin was used (Cicerale et al. forthcoming). Briefly, oleocanthal was extracted from the oil matrix by liquid–liquid partitioning. The solvent containing the oleocanthal extract was collected and evaporated, leaving the dried extract to be dissolved in methanol–water (v:v, 1:1). This methanol–water phase containing oleocanthal was then analyzed by HPLC (Impellizzeri and Lin 2006; Cicerale et al. forthcoming).

Experiment 1—location of oleocanthal irritation

Materials and methods

Anecdotal evidence suggests that oleocanthal irritation is localized to the back of the throat. However, to date, no studies have examined this. A within-subjects design was used to examine the location of oleocanthal irritation in the oral cavity. Twenty subjects (14 women, mean age 33.7 ± 10.5 years) were recruited from Melbourne, Australia. Subjects gave their written informed consent prior to participation on an approved Institutional Review Board form (EC253-2006). All testing took place in the Sensory Laboratory at Deakin University. Subjects were required to attend 2 training and 3 test sessions. Subjects were asked to refrain from consuming food and drink (except room temperature water) and use of chemesthetic agents (toothpaste, mouthwash, and gum) 2 h prior to testing.

Subjects were trained in the use of the general labeled magnitude pseudologarithmic scale (gLMS) following the published standard procedures by Bartoshuk et al. (2004) (Green et al. 1993, 1996). The gLMS is a labeled scale of sensation intensity that requires subjects to rate perceived intensity along a vertical axis containing the adjectives: barely detectable = 1.5, weak = 6, moderate = 17, strong = 35, very strong = 52, and strongest imaginable sensation of any kind = 100. The adjective placement was derived experimentally and yields data equivalent to magnitude estimation. Only the adjectives, and not their corresponding numbers, are visible to the subjects. The experimenter receives numerical data from the scale (Green et al. 1993, 1996; Bartoshuk et al. 2004).

Subjects were also educated on what the oropharynx was. In the training, subjects were told that the researchers were trying to determine if oleocanthal elicits irritation generally in the mouth or at specific sites. They were not told about anecdotal evidence that suggests that oleocanthal elicits irritation solely in the oropharynx.

Oleocanthal-containing EVOO (54 mg/kg) was supplied by Redisland Australia (Braeside, Australia), and soda water was supplied by Kirks Classics (Melbourne, Australia). All testing took place in a specialized sensory testing facility comprising 7 individual booths. Each subject was isolated from other subjects by vertical dividers to eliminate interaction between subjects. Subjects also wore noseclips to eliminate olfactory cues.

After subjects were familiarized with the scale, they were given hypothetical stimuli and asked to rate their intensity on the scale. Feedback was given by the researchers as to where the general population rated those stimuli for intensity, helping the subjects to better understand how the scale should be used. After this, the subjects were supplied with references for barely detectable (sweetness of a 50 mM sucrose solution), weak (warmth of lukewarm water), and moderate (irritation of carbonated soda water) to evaluate and rate on the scale. For strong, very strong, and strongest imaginable, subjects were given hypothetical examples. Subjects were also trained to evaluate the irritation intensity of oleocanthal (54 μg/g) in EVOO and CO_2 in soda water. Subjects were given 3 samples of each to rate. If an individual's rating was too variable (i.e., more than 25% out of mean value) or they were not using the scale correctly, the subject was removed.

An aliquot of 5 ml of EVOO and 15 ml of soda water (for oropharyngeal testing) and 15 ml of both EVOO and soda water (for anterior tongue and anterior mouth testing) were presented in 30-ml polyethylene medicine cups (McFarlane Medical, Surrey Hills, Australia). Subjects rinsed their mouths with filtered (FI) water (8 μm particulate filter with an activated charcoal filter, Dura®) at least 3 times over a 2-min period before commencement of testing. Each subject sampled and rated (using the gLMS) EVOO for oleocanthal irritation and soda water for CO_2 irritation in the oropharynx, anterior tongue, and anterior mouth in duplicate (see Figure 1 for diagram of the oral cavity). In each of the 3 test sessions, 2 olive oil and 2 soda water samples were presented in a randomized order with an interstimulus interval of 1 min between samples.

For evaluation of oleocanthal irritation in the oropharynx, the method of sensory evaluation was adapted from Beauchamp et al. (2005). Subjects were required to place the oil in their mouths and tilt their head back to allow the oil to drizzle down the back of their throat. Subjects were

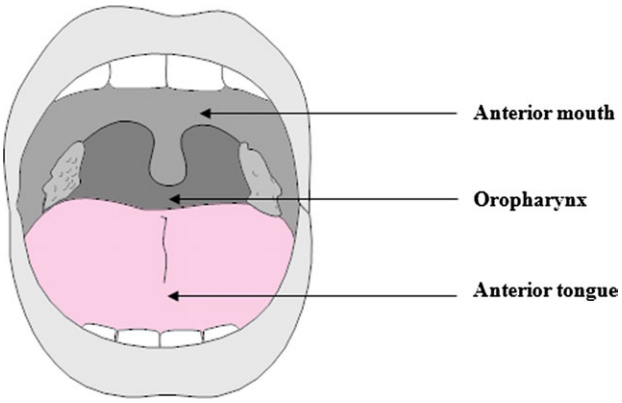

Figure 1 The oropharynx, anterior tongue, and anterior mouth.

asked to keep the oil at the back of the throat for ~5 s, then swallow the sample in 2 stages, and rate the peak intensity of irritation after 20 s. Swallowing the sample in 2 stages meant that the subject swallowed the oil and then immediately swallowed again ensuring the oropharynx was coated with the oil. For evaluation of CO_2 irritation in the oropharynx, subjects were asked to gargle the sample at the back of their throats for 5 s, swallow the sample, and then rate peak intensity of irritation after 20 s. For the anterior tongue, a tongue dip method was used for both stimuli, where subjects were asked to place their tongue in the sample for 5 s, take their tongue out of sample, and rate the peak intensity of irritation after 20 s (Keast and Breslin 2002). For the anterior mouth, subjects were asked to rinse both stimuli in their mouth for 5 s, spit, and then rate the peak intensity of irritation after 20 s. All evaluations were performed in duplicate.

Data analysis

Data were analyzed using SPSS for Windows, version 14.0. One-way repeated measures analysis of variance (ANOVA) with Bonferroni correction was used to determine if a difference in perceived oleocanthal irritation existed between the oropharynx, anterior tongue, and anterior mouth.

Results

Oleocanthal irritation was greater in the oropharynx compared with the anterior tongue or anterior mouth (Figure 2) [Wilks' Lambda = 0.07, $F(2,38) = 265.70$, $P < 0.05$]. No significant difference in irritation was observed between the anterior tongue and the anterior mouth ($P = 1.00$). CO_2 irritation was perceived equally at all 3 sites of the oral cavity [Wilks' Lambda = 0.07, $F(2,38) = 2.08$, $P = 0.14$].

Experiment 2—test–retest reliability

Materials and methods

Test–retest reliability of oleocanthal irritation ratings was conducted to determine the reproducibility and thus reliability of such ratings. Materials and methods are equivalent to those in experiment except as otherwise stated. A within-subjects design was used to examine the test–retest reliability of oleocanthal irritation intensity ratings. Thirteen subjects (10 women, mean age 32.7 ± 10.4 years) were recruited from Melbourne, Australia. Subjects were required to attend 2 training and 6 test sessions.

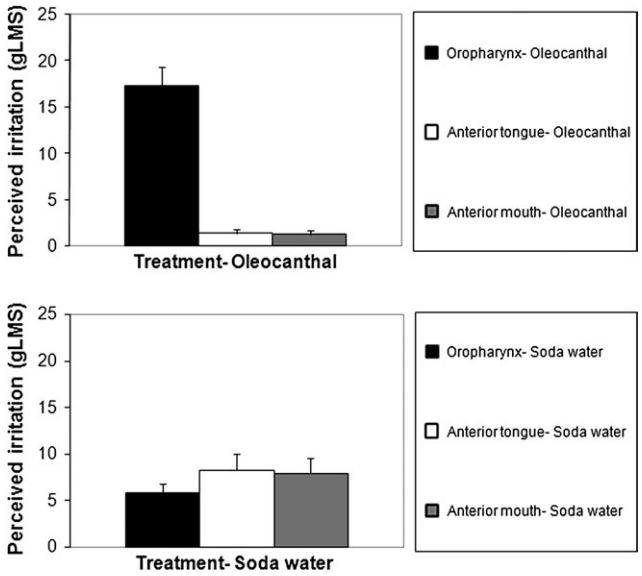

Figure 2 Bar graph depicting gLMS ratings (mean ± standard error) for intensity of oleocanthal and CO_2 irritation on the oropharynx, anterior tongue, and anterior mouth.

Subjects were trained to evaluate the irritation intensity of oleocanthal in EVOO, irritation intensity of CO_2 in soda water, and intensity of sweetness of sucrose. EVOO containing 54 mg/kg of oleocanthal, soda water, and a 200 mM sucrose solution were used in the experiment. The soda water and sucrose solutions were included in the experiment as control stimuli. Sucrose was supplied by pure Australian white sugar resources, and FI water was used to make the sucrose solution.

Aliquots of 5 ml of oil and 15 ml of soda water and sucrose solution were presented in 30-ml polyethylene medicine cups. In any one session, one sample of EVOO, soda water, and sucrose solution was evaluated with an interstimulus interval of 1 min between samples. Each stimulus was evaluated on 6 separate occasions. For the evaluation of CO_2 irritation in the anterior mouth and sweetness of sucrose, subjects were asked to rinse both stimuli in their mouth for 5 s, spit, and then rate the peak intensity of irritation (for CO_2) and sweetness (for sucrose) after 20 s.

Data analysis

A Pearson's product–moment coefficients correlation was conducted between the averaged values of the first 3 and the last 3 ratings of each of the stimuli to determine oleocanthal, CO_2, and sucrose test–retest reliability. A paired-samples

t-test was also conducted to establish if there was a statistically significant difference between the averaged values of the first and last 3 ratings of each of the stimuli.

Results

Correlation analysis revealed highly reproducible CO_2 (r = 0.94, n = 13, $P < 0.05$) and sucrose (r = 0.98, n = 13, $P < 0.05$) ratings for all subjects. Test–retest reliability of oleocanthal ratings revealed a slightly weaker correlation (r = 0.61, n = 13, $P < 0.05$) than that for CO_2 and sucrose. Refer to Table 1 for subject gLMS rating (mean ± standard error) for each stimulus. Furthermore, there was no statistical significant difference between the averaged values of the first 3 and the last 3 ratings of each of the stimuli ($P > 0.05$).

Experiment 3—time–intensity profile of oleocanthal oropharyngeal irritation

Materials and methods

The time–intensity profile of oleocanthal oropharyngeal irritation was examined to determine the time at which irritation is most intense. This information will aid with the establishment of methods for future studies that utilize the sensory-directed approach for the determination of oleocanthal concentration in EVOOs. Materials and methods are equivalent to those in experiment 1 except as otherwise stated.

A within-subjects design was used to examine the time–intensity profile of oropharyngeal irritation of oleocanthal. Thirteen subjects (10 women, mean age 23.0 ± 4.0 years) were recruited from Philadelphia, PA. Subjects gave their written informed consent prior to participation on an approved Institutional Review Board form (Setbapp5005). Subjects were required to attend 10 training and 9 test sessions. The 10 training sessions were used to familiarize subjects with the stimulus and rating oleocanthal irritation over a period of time. They were also used to obtain consistency in ratings. In each of the 9 test sessions, one olive oil sample was presented and irritation was rated over a period of time.

Oleocanthal-containing EVOO (43 μg/g) was supplied by Lucini Italia (Bolgheri Tuscany, Italy), and corn oil was purchased from Wholefoods Supermarket (Philadelphia, PA). Corn oil was used as a diluent to reduce the level of oleocanthal in the EVOO. Three levels of dilution were used: 100% EVOO, 0% corn oil; 75% EVOO, 25% corn oil; and 50% EVOO, 50% corn oil.

An aliquot of 3.5 ml of oil was presented in 30-ml polyethylene medicine cups. Subjects were asked to rate the intensity of irritation at the oropharynx elicited by the EVOO and EVOO-corn oil mixtures on the gLMS. Subjects were given an unidentified sample of oil, required to swallow the sample in 2 stages, and then rate the intensity of throat irritation at 9 time points over a 3-min period (0, 5, 15, 30, 45, 60, 90, 120, and 180 s). All evaluations were performed in triplicate, and presentation order of oils was randomized.

Data analysis

A 2-way ANOVA was used to determine if there was a significant main effect of time and concentration on oleocanthal irritation. If a significant difference was detected, multiple *t*-tests with Bonferroni correction were carried out.

Results

The temporal pattern of oropharyngeal irritation from oleocanthal is shown in Figure 3. Results from a 9 by 3 (time × concentration) 2-way ANOVA of EVOO revealed that there was a significant main effect of time [$F(8,18) = 9.5$, $P < 0.001$]

Table 1 Subject gLMS rating (mean ± standard error [SE]) for EVOO, soda water, and sucrose

Subject number	EVOO containing 54 μg/g oleocanthal (mean ± SE)	Soda water (mean ± SE)	200 mM sucrose (mean ± SE)
1	11.8 ± 0.9	15.4 ± 0.3	15.3 ± 0.3
2	24.5 ± 3.6	23.8 ± 1.4	18.4 ± 0.6
3	14.1 ± 3.3	18.3 ± 0.4	11.3 ± 0.7
4	16.7 ± 4.1	23.8 ± 1.3	13.5 ± 1.0
5	25.3 ± 2.6	16.3 ± 0.0	5.3 ± 0.3
6	22.0 ± 1.3	22.4 ± 0.5	12.6 ± 0.7
7	14.5 ± 1.6	17.9 ± 0.3	14.2 ± 0.2
8	13.7 ± 1.7	16.3 ± 0.4	6.0 ± 0.2
9	15.9 ± 3.0	16.9 ± 0.3	10.2 ± 0.4
10	14.8 ± 4.4	17.2 ± 0.2	12.5 ± 0.4
11	15.4 ± 3.1	17.8 ± 0.2	9.7 ± 0.3
12	20.8 ± 1.7	16.3 ± 0.0	5.8 ± 0.0
13	12.3 ± 3.8	20.0 ± 0.9	18.2 ± 0.6

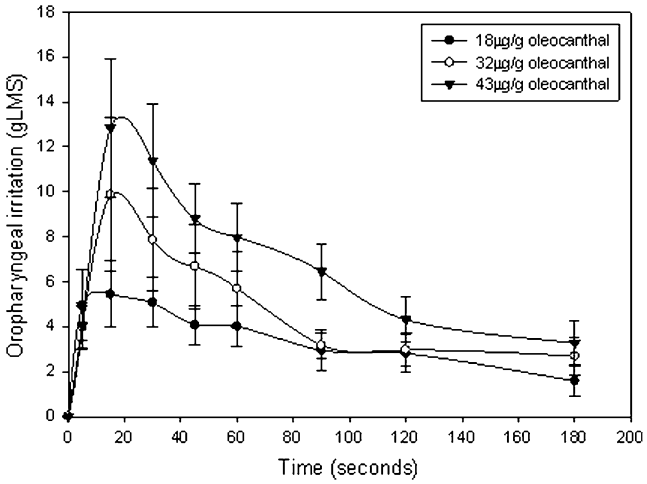

Figure 3 Temporal profile of oleocanthal irritation.

and concentration [$F(2,24) = 6.9$, $P < 0.05$] at 15 s postexposure. There was no interaction between time and concentration [$F(16,10) = 0.8$, $P = 0.6$]. Post hoc pairwise tests demonstrated that the intensity of irritation from all 3 concentrations of oleocanthal at 180 s was significantly more than at time zero ($P < 0.05$), indicating that duration of sensation exceeded 180 s. Peak irritation was perceived at 15 s postexposure and lasted over 180 s.

There were significant differences in irritation intensity between the highest (43 μg/g) and lowest (18 μg/g) oleocanthal concentration time intensity curves ($P < 0.05$). The 32-μg/g oleocanthal time intensity curve was not significantly different from 43-μg/g ($P = 0.6$) and 18-μg/g ($P = 0.9$) time intensity curves.

Experiment 4—individual variation in oleocanthal oropharyngeal irritation

Materials and methods

This experiment was conducted to determine the extent of variation in perceived intensity of oleocanthal irritation among the general population. Materials and methods are equivalent to those in experiment 1 except as otherwise stated. A between-subjects design was used to examine individual variation in oleocanthal oropharyngeal irritation intensity. The experiment was carried out on 2 separate occasions with 2 different population groups. Both groups of subjects were required to attend one session, which consisted of training and testing.

Group A—Subjects ($n = 50$, 40 women, mean age 23.0 ± 5.0 years) were recruited from Philadelphia, PA. Subjects were asked to rate the irritation intensity of oleocanthal-containing EVOO (154 μg/g) (Laudemio, Tuscany, Italy) on the gLMS. As a control irritant, subjects were asked to rate the intensity of mouth irritation elicited by soda water. Group B—Subjects ($n = 84$, 76 women, mean age 20.7 ± 3.7 years) were recruited from Melbourne, Australia. Subjects were asked to rate the irritation intensity of oleocanthal-containing EVOO (70 μg/g) on the gLMS. As a control stimulus, subjects were asked to rate the intensity of sweetness elicited by a 200-mM sucrose solution.

Group A subjects were given 3.5 ml of oleocanthal-containing EVOO and 10 ml of soda water in 30-ml polyethylene medicine cups. Subjects were instructed to rate the peak intensity of irritation in the oropharynx for oil and anterior mouth for soda water. All evaluations were made in triplicate. For group B, subjects were given 5 ml of oleocanthal-containing EVOO and 15 ml of a 200-mM sucrose solution in 30-ml polyethylene medicine cups. Subjects were instructed to rate the peak intensity of oropharyngeal irritation for oil and intensity of sweetness elicited by sucrose.

Data analysis

Pearson's product–moment coefficients correlation were also conducted to analyze the relationship between oleocanthal irritation intensity, CO_2 irritation intensity, and the sweetness of sucrose intensity. Mean, range, and variance values were used to determine variability in perceived oleocanthal irritation among individuals. P values <0.05 were considered significant.

Results

Group A results demonstrated a greater mean, range, and variance in perceived intensity of oropharyngeal irritation from oleocanthal-containing EVOO ($n = 50$, mean gLMS 25.2, range gLMS 10–49, variance 79.1) compared with anterior oral irritation from CO_2 ($n = 50$, mean gLMS 10.3, range gLMS 5–14, variance 3.6). Figure 4 shows respective histograms with an overlay of a normal distribution. There was no correlation between perceived intensity of oleocanthal irritation and soda water irritation ($r = –0.15$, $n = 50$, $P = 0.92$), indicating a lack of a shared mechanism between the stimuli.

Group B results also demonstrated a greater mean, range, and variance in perceived intensity of oropharyngeal irritation from EVOO ($n = 84$, mean gLMS 24.0, range gLMS 1–81, variance 243.3) compared with the intensity of sucrose sweetness ($n = 84$, mean gLMS 9.8, range gLMS 0–34, variance 45.0). There was no correlation between oleocanthal oropharyngeal irritation and sucrose sweetness intensity ratings ($r = 0.17$, $n = 84$, $P = 0.12$), indicating that irritation was either independent of an individual's idiosyncratic use of the gLMS or is a result of an overall effect like individual's overall oral sensitivity. Figure 5 shows respective histograms with an overlay of a normal distribution.

General discussion

This study demonstrated that oleocanthal irritation is greatest in the oropharyngeal region of the oral cavity, and the irritation produced is not correlated with that of CO_2 irritation and therefore does not elicit irritation via a generalized acid-sensing mechanism. These findings may be a result of the existence of chemesthetic receptors located in the oropharyngeal region that respond specifically to the natural nonsteroidal anti-inflammatory compound, oleocanthal. Alternatively, it remains possible that oleocanthal activates other receptors in the throat.

The localized irritation of oleocanthal is surprising in light of the fact that nociceptive neurons typically respond to most irritants (Green 2004). For instance, despite slight regional differences in chemical irritant sensations, capsaicin (Green and Hayes 2003), and menthol evoke irritant sensations throughout the oral cavity and other mucus membrane areas of the body. Chemical irritation of a restricted area (as in the case of oleocanthal) is rare (Green 2004). Similarly, ibuprofen solely irritates the oropharynx, suggesting that this mucosal region possesses sensory receptors specific to compounds structurally related to oleocanthal and ibuprofen (Breslin et al. 2001).

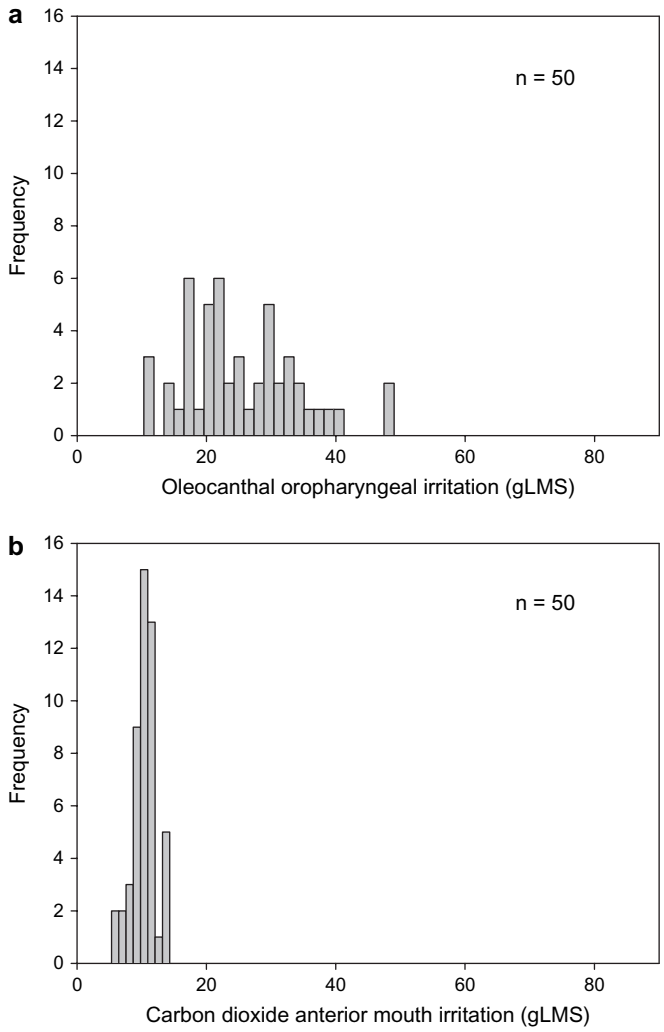

Figure 4 Histograms of rating frequency (gLMS) of irritant intensity of oleocanthal and CO_2. The *x* axis represents the average irritation on the gLMS by an individual subject. The *y* axis represents the number of subjects. Each bar represents the number of people who rated the irritation at the specified intensity range.

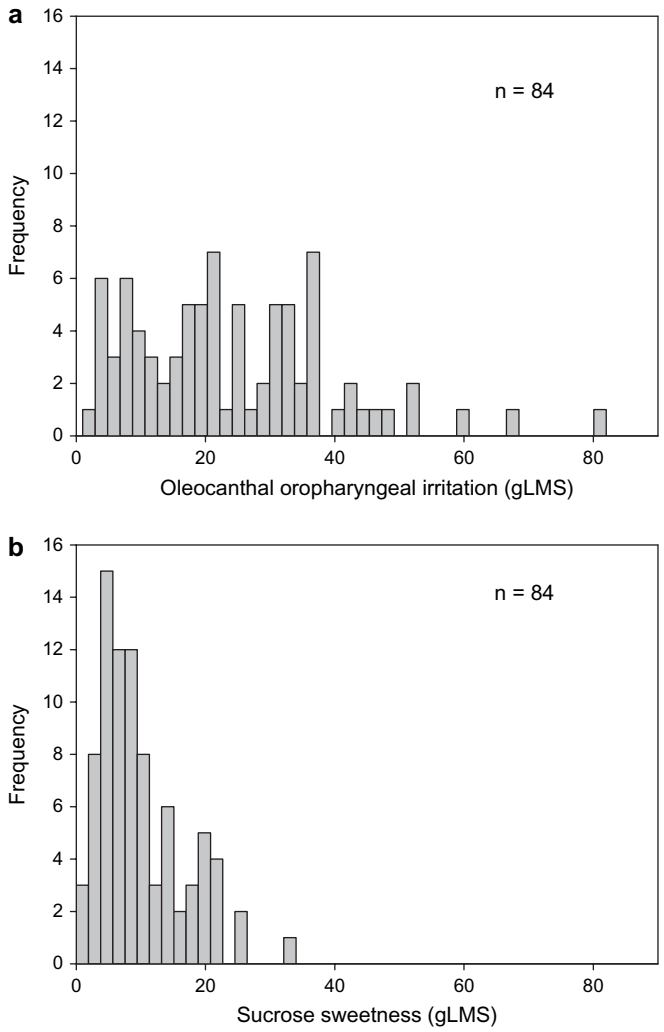

Figure 5 Histograms of rating frequency (gLMS) of irritant intensity of oleocanthal and sweetness of sucrose. The *x* axis represents the average irritation on the gLMS by an individual subject. The *y* axis represents the number of subjects. Each bar represents the number of people who rated the irritation at the specified intensity range.

All subjects gave reproducible ratings for both CO_2 mouth irritation and sucrose sweetness. Oleocanthal oropharyngeal irritation ratings were less reliable than for CO_2 and sucrose but are nevertheless somewhat reliable $r = 0.61$. The causes of this lower reproducibility for oleocanthal oropharyngeal irritation ratings are not entirely clear. The difference in reproducibility between CO_2 and oleocanthal irritation ratings may be due to differences in diffusion through the epithelium. Furthermore, differences in salivary composition from test to test (Breslin et al. 2001) and thickness of the mucus layer present at the back of the throat at time of testing might affect oleocanthal ratings. Further research is required to explore the factors that affect the variability in perceived oleocanthal oropharyngeal irritation. Nevertheless, the reliability was sufficiently high to yield meaningful data.

There was variability among subjects in perceived irritation from oleocanthal. Such individual variation in perception of oleocanthal may be related directly to the specific form and quantity of receptors in the oral cavity, as has been reported with other oral stimuli such as 6-*n*-propylthiouracil and phenylthiocarbamide bitterness (Bufe et al. 2005; Hansen et al. 2006). There were nonsignificant correlations between ratings of oleocanthal irritation and the irritation of CO_2 or the sweetness of sucrose. Thus, the large variability in perceived intensity of oleocanthal should not be attributed to an individual's idiosyncratic use of the gLMS. This indicates that the irritation elicited by oleocanthal and the irritation elicited by CO_2 access somewhat different physiological mechanisms. Similar to the findings of Breslin et al. (2001) regarding ibuprofen, the large interindividual variation and noncorrelation with CO_2 intensity suggest that

oleocanthal irritation may be due to specific receptors in the oropharynx that differ from person to person in their density or their ability to bind and respond to oleocanthal.

A limitation of the study to consider was that the majority of participants were women, and therefore, the results obtained may not generalize to men. Future studies could include a greater proportion of men to investigate if there are gender differences regarding the perceptual attributes of oleocanthal.

In summary, oleocanthal irritation was localized to the oropharyngeal region of the oral cavity and is highly variable among individuals. Taken together, our findings suggest that chemical-specific receptors are located in the oropharyngeal region of the oral cavity that respond to oleocanthal.

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