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Oral Cavity Discrimination of Vapor-Phase Long-Chain 18-Carbon Fatty Acids

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Abstract

Linoleic, oleic, and stearic fatty acids, presented vapor-phase retronasally, were discriminable from blanks and each other, but the same concentrations, oral-cavity-only (OCO), were not discriminable from blanks. It remained possible that higher concentrations might be discriminable OCO. To evaluate this, participants attempted to discriminate undiluted linoleic, oleic, or stearic acids, vapor-phase OCO, from blanks. For each fatty acid, participants received 5 stimulus delivery containers (SDCs) in 2 trials; 4 SDC held blanks, the fifth, a fatty acid. As a "positive control" in 2 trials, participants received vapor-phase OCO peppermint extract and blanks. For all trials, the task was to select the 1 different SDC. It was found that the 1 different SDC was selected in 24% of stearic, 32% of linoleic, 47% of oleic acid, and in 92% of peppermint trials; discriminations (the 1 different SDC selected in both trials) occurred in 0%, 16%, 26%, and 84% of pairs, respectively. Correct selections for oleic acid differed from chance, $P = 0.0004$, but not for linoleic acid, $P = 0.125$, or stearic acid, $P = 0.345$, Bonferroni corrected. Vapor-phase oleic acid can be an oral cavity trigeminal stimulus, linoleic acid might be (uncorrected $P = 0.0384$), but vapor-phase stearic acid cannot be.

Key words: 18-carbon fatty acids, human, long-chain fatty acids, oral cavity, smell, trigeminal

Introduction

Vapor-phase chemicals are potential stimuli for both olfactory receptor neurons and trigeminal sensory nerve branches in terrestrial mammals. The relationship between olfactory and trigeminal sensitivity is nonsymmetrical. It is generally believed that many vapor-phase trigeminal stimulus chemicals will also be olfactory stimuli (e.g., Cain and Murphy 1980; Brand 2006; Lombion et al. 2009) but with the olfactory stimulation typically occurring at appreciably lower concentrations than those necessary for stimulation of trigeminal branches. It should be noted that certain trigeminal stimuli, such as CO_2, are thought to produce little (e.g., Melzner et al. 2011) or no (see Cain et al. 2006) olfactory stimulation in humans. However, several vapor-phase olfactory stimuli (e.g., vanillin and phenylethyl alcohol) do not stimulate trigeminal sensory nerve branches sufficiently for human perceptual responses (Doty et al. 1978; Radil and Wysocki 1998; Wysocki and Wise 2004; Cometto-Muñiz et al. 2005), including those innervating the oral cavity (e.g., Chen and Halpern 2008; Stephenson and Halpern 2009). Consequently, if specific vapor-phase stimuli are found to be effective when presented orthonasally or retronasally, it does not necessarily follow that these stimuli will be effective when restricted to the oral cavity.

The presence or absence of oral cavity responses to vapor-phase chemicals is of particular interest when such chemicals are likely components of food systems. One such set of chemicals is long-chain 18-carbon fatty acids. These fatty acids, specifically linoleic, oleic, and stearic fatty acids, are components of common edible oils (Gunstone 2002) and of some fruits (e.g., Santos et al. 2011; Villa-Rodríguez et al. 2011). They can be smelled both orthonasally and retronasally (Chalè-Rush et al. 2007a; Bolton and Halpern 2010; Kallas and Halpern 2011) but have failed to produce oral-cavity-only (OCO) responses when delivered in vapor phase (Bolton and Halpern 2010). One possible reason for the reported lack of OCO responses to 18-carbon fatty acids is the low concentrations that were employed.

The goal of the present research was to examine OCO discrimination of undiluted vapor-phase 18-carbon long-chain fatty acids that had previously been discriminated from blanks orthonasally and retronasally but not OCO. This approach differs from previous studies that had used diluted vapor-phase long-chain fatty acids.

The hypothesis was that OCO discrimination of vapor-phase 18-carbon fatty acids would not occur. This hypothesis was based upon the prior observation that diluted vapor-phase 18-carbon fatty acids had not been discriminated OCO (Bolton and Halpern 2010). Because a failure to find discrimination could be due to factors other than the stimuli, vapor-phase presentations of peppermint extract were included to serve as a positive control. Based upon previous data, it was expected that OCO discrimination of the vapor-phase peppermint extract would occur (Dragich and Halpern 2008; Stephenson and Halpern 2009). If the nondiscrimination hypotheses for vapor-phase 18-carbon fatty acids were confirmed, this result would indicate that the oral cavity component of the trigeminal sensory system cannot respond to vapor-phase linoleic, oleic, and stearic long-chain fatty acids. This finding would suggest that responses to vapor-phase presentations of these food-related fatty acids are dependent upon the sensory systems available in the nasal cavities. Conversely, rejection of the nondiscrimination hypotheses for one or more of the higher concentration vapor-phase 18-carbon fatty acids that are employed would indicate that both the oral cavity trigeminal system and the nasal cavity sensory systems can respond to vapor-phase 18-carbon fatty acids and that responses to these food-related fatty acids in vapor phase may begin in the oral cavity. OCO discrimination of vapor-phase peppermint extract would confirm that the stimulus delivery and response procedures will support oral cavity discrimination responses for some vapor-phase stimuli.

Materials and methods

Participants

Participants were 19 paid volunteers, 12 females, and 7 males. Participants' ages ranged from 18 to 22 years (median age ± semi-interquartile range [SIR] = 19 ± 1.0 years). They were nonsmoking, nonpregnant, and nonlactating individuals associated with Cornell University, who could communicate in American English. Participants were recruited using flyers that were posted around Cornell University's Ithaca NY campus, with an online website (http://susan2.psych.cornell.edu/) and with interpersonal contacts. These were the only exclusion and inclusion criteria used. No chemosensory screening of participants was done (it should be noted that correct selection of the peppermint extract on at least 1 trial was required for participants to continue in the study; see stimulus discrimination testing). The protocol was reviewed and approved by Cornell University's Institutional Review Board for Human Participants (IRB). Each potential participant read the IRB-approved informed consent form, asked any questions they had, and, if they decided to participate in the study, signed the approved informed consent form. Participants were asked not to eat or drink anything except water for 1 h before a scheduled session. Participants were informed that the experiment would test their ability to smell.

Stimuli

The 3 fatty acid stimuli used were 1) linoleic acid (CAS# 60-33-3, food/pharmaceutical grade, Kosher, 81% lot assay, Penta Manufacturing Co. #12-64000), 2) oleic acid (CAS#112-80-1, Food Chemicals Codex [FCC], food grade, Kosher, Sigma-Aldrich W281506), and 3) stearic acid (CAS# 57-11-4, FCC, Halal, ≥95%, Sigma-Aldrich W303518). All fatty acid stimuli were used undiluted.

The peppermint extract was an alcohol-free food grade liquid extract of plant material (organic sunflower oil and organic peppermint oil), identified as a flavor, and sold for inclusion in human foods and beverages (Frontier Natural Products Co-op). The peppermint extract was "Quality Assurance International" certified organic and "Kosher Supervision of America" certified Kosher. Peppermint extract was selected as the positive control stimulus because it had been discriminated in previous studies of OCO responses to vapor-phase stimuli (Dragich and Halpern 2008; Stephenson and Halpern 2009). It was used undiluted.

The blank for the liquid fatty acids (linoleic and oleic) was United States Pharmacopeia mineral oil; for the solid fatty acid (stearic), NaCl (analytical reagent grade). NaCl was employed as the blank for stearic acid because they are both solids at 21 ± 1 °C, thus preventing a discrimination based upon the dynamic characteristics of the stimulus delivery containers (SDCs) while avoiding the introduction of another vapor-phase stimulus. The blank for the peppermint extract was sunflower oil ("Organic high heat sunflower oil". Spectrum brand).

The linoleic and oleic acids were kept in the dark under prepure nitrogen at 4.5 °C. Stearic acid was stored in the dark at –18.5 °C. The United States Pharmacopeia mineral oil was stored in the dark at room temperature. The peppermint extract and the sunflower oil were stored in the dark at 4.5 °C. NaCl was stored in a desiccator in the presence of $CaCl_2$. All stimuli were brought to room temperature, 21 ± 1 °C, before use.

Each presentation of a stimulus or its blank in a SDC (see Stimulus delivery containers) had a volume of 5 mL for the liquid stimuli and liquid blanks or a weight of 2 g for the solid stimuli (stearic acid [volume of 2.4 mL] and NaCl [volume of 0.9 mL] blank). In previous studies, 5 mL of diluted liquid fatty acids (linoleic or oleic) or 2 g of stearic acid had been sufficient to permit orthonasal and retronasal detection of the fatty acids when presented in vapor phase (Bolton and Halpern 2010) and retronasal discrimination between them (Kallas and Halpern 2011). In both the previous and the present study, the stimuli and blanks just covered the bottom of the SDC.

Stimulus delivery containers

Vapor-phase stimuli were presented at 21 ± 1 °C in 118 mL, 5.1-cm high black homopolymer polypropylene elliptical containers having the shape of a frustum of an ellipsoid (Ellipso Portion Cups, Newspring Packaging; see Chen and Halpern 2008; Parikh et al. 2009; Stephenson and Halpern 2009; Bolton and Halpern 2010; Kallas and Halpern 2011). The containers had tight-fitting homopolymer polypropylene transparent lids (Ellipso Portion Cups, Newspring Packaging) (Chen and Halpern 2008; Stephenson and Halpern 2009; Parikh et al. 2009; Kallas and Halpern 2011), with two 5-mm holes made along its major axis. A 6.5-cm long, 5-mm outer diameter, 4.8-mm inner diameter homopolymer polypropylene straw (Jetware unwrapped plastic drinking straw, Jet Plastica Industries, Inc.) was inserted 3.25 cm through 1 hole, perpendicular to the lid. Aluminum foil was wrapped on the lids to mask the SDC's contents. The black elliptical container plus aluminum-wrapped lid with holes and inserted straw constituted the SDC used in this study to present the fatty acid stimuli, the peppermint extract stimulus, and their blanks (mineral oil paired with liquid fatty acids linoleic and oleic; NaCl paired with solid fatty acid stearic; sunflower oil paired with ''positive control'' peppermint extract). The straw sampled the headspace above the liquid or solid stimuli and blanks. This container had been named ''odorant delivery container'' in Chen and Halpern (2008), Stephenson and Halpern (2009), and Parikh et al. (2009), with the acronym ODC. In Kallas and Halpern (2011) and the present study, the term ''stimulus delivery container'' and the acronym SDC are used because of the possibility of trigeminal stimulation.

Vapor-phase concentrations in the SDC for linoleic, oleic, and stearic fatty acids were calculated using software that employs the Hass–Newton equations for vapor-phase partial pressure (ACD/ChemSketch, Advanced Chemistry Development) and extrapolated to 21 °C, the temperature at which the fatty acids were presented. At that temperature, all three 18-carbon long-chain fatty acids would have vapor pressures (partial pressures) of approximately 0.01 Pa. Concentrations in parts per million were calculated to be approximately 71 ppm for the vapor-phase components of the 3 fatty acids.

For the SDC used in trials involving stearic acid and NaCl, a 2.54-cm × 2.54-cm Kimwipe (low-lint, low-extractable scientific wipe; Kimberly-Clark) square was taped around the straw end inside the container to prevent possible particulate inhalation.

Nose clip

All stimulus presentation sequences began with the participant wearing a disposable Spiro nose clip (Nose Clip D1060, Spirometrics). The nose clip remained on throughout each OCO trial. Each nose clip was used for 1 participant and then discarded.

Stimulus discrimination testing

In each trial, participants received a set of 5 SDC. Four SDC had the same content; 1 was different. Participants were instructed to select the 1 different SDC from the other 4 SDC of the set. The location of the SDC containing the 1 different stimulus was randomized across participants. Two successive sets presented SDC with the same stimulus chemical and blanks but differing in the location of the 1 different stimulus–containing SDC in the row. For the 2 sets of each fatty acid, a fatty acid was in 1 SDC and a blank was in the 4 other SDC. In positive control trials, the SDC contained either Frontier Natural peppermint flavor extract or sunflower oil; discrimination was expected for positive control trials. In all trials, participants were permitted to resample an SDC if they wished. However, once the 1 different SDC had been selected, the selection could not be changed.

All participants received the pairs of stimuli and blanks presentations in the same order: peppermint extract, linoleic fatty acid, oleic fatty acid, and the stearic fatty acid. Participants were encouraged to not rush between pairs of stimuli and blanks. Approximately 30 s elapsed between the selection of the 1 different SDC by a participant and the presentation of the next 5 SCD. Participants who failed to select the 1 different peppermint extract SDC on at least 1 of the 2 presentations were thanked for their participation and excused. Of 20 participants who began the study, 19 met this criterion. The 1 individual who did not select the 1 different peppermint extract SDC on at least 1 of the 2 presentations was the second individual tested.

Before trials began, an experimenter demonstrated OCO sampling using an empty SDC; a second empty SDC was available if the participant wished to practice. It was explained that OCO smelling was inhaling vapor-phase stimuli into the oral cavity using the straw of an SDC and exhaling from the oral cavity, with the nose clip remaining in place. Participants were shown that the SDC was to be held approximately horizontally.

Statistical analyses

Because each fatty acid and the peppermint extract were presented twice within the 8 presentations of 5 SDC sets, each participant could select the SDC containing a particular fatty acid or the peppermint extract a total of 0, 1, or 2 times across these trials. The number of times that each of the 19 participants correctly selected the SDC containing the 1 different SDC, for each of the 3 fatty acids and the peppermint extract, is shown in Table 1. These numbers are the raw data of this study. Central tendencies and variability of correct selections were obtained by calculating medians and SIRs for each stimulus across all 19 participants (Table 1). The criterion for discrimination of a fatty acid or the peppermint extract by a participant was established as correct selection of the SDC containing a particular stimulus a total of 2 times across the 2 presentations of that stimulus. This

Table 1 The number of correct selections of the 1 SDC that contained a fatty acid or peppermint extract (the positive control) from the 5 SDC that were presented in each of 2 trials for each stimulus, for each participant and stimulus, and the median number of correct selections, and SIR, for each stimulus

Participant	Stimuli			
	Oleic acid	Linoleic acid	Stearic acid	Peppermint extract
1	1	0	1	**2**
2	0	1	0	**2**
3	1	0	0	**2**
4	1	0	1	**2**
5	1	0	0	1
6	0	0	0	1
7	**2**	1	0	**2**
8	0	0	1	**2**
9	0	0	1	**2**
10	0	0	1	**2**
11	1	1	1	**2**
12	0	0	0	**2**
13	**2**	**2**	0	**2**
14	**2**	1	0	1
15	**2**	1	0	**2**
16	1	**2**	1	**2**
17	1	0	1	**2**
18	**2**	1	0	**2**
19	1	**2**	1	**2**
Median	1	0	0	2
SIR $(Q_3 - Q_1)/2$	0.75	0.5	0.5	0

Bold-faced numbers denote selection of the SDC containing that stimulus by that participant on both of the possible instances. Such correct selection has a probability of 0.04 and was taken as the criterion for discrimination.

criterion was selected because the probability of selecting by chance the 1 SDC containing a fatty acid or the peppermint extract from the 5 SDCs presented in each set was 0.2. For both presentations of a given stimulus, the probability of selecting by chance the correct SDC was $0.2 \times 0.2 = 0.04$. Overall percent discriminations for each stimulus across all participants were calculated by counting the number of participants who discriminated that stimulus (Table 1), dividing by the number of participants and multiplying by 100 (Figure 1). This provided an overall indication of the degree of discriminability for each stimulus.

For inferential statistics, because of the relatively small sample size and in order to avoid unnecessary assumptions, nonparametric binomial tests were used, with $P \leq 0.05$ taken as an indication of statistical significance. A binomial test

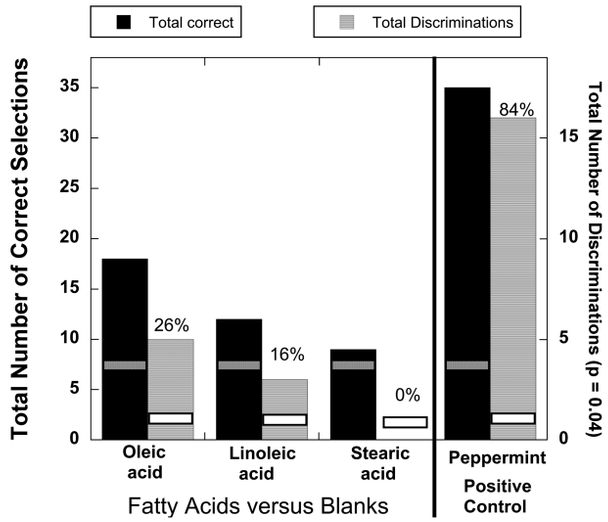

Figure 1 Number of correct selections of the 1 different SDC of 5 SDC presented OCO twice to 19 participants (totals of 38 possible correct selections) (solid columns; left axis) and the number of discriminations (selecting the 1 different SDC on both trials [totals of 19 possible discrimination], for which the probability by chance = 0.04) (horizontal striped columns; right axis), for each of 4 types of vapor-phase presentation comparisons: fatty acids versus blanks = linoleic acid versus mineral oil blanks, oleic acid versus mineral oil blanks, and stearic acid versus NaCl blanks; positive control = peppermint flavor extract versus sunflower oil blanks. Percentages above the discrimination columns are the percentages of participants who discriminated each fatty acid or the peppermint extract. The filled rectangles in the "number of correct selections" columns at 7.6 correct selections represent the number of correct selections that would be expected to occur by chance. The open white rectangles at the "number of discriminations" columns at 0.76 discriminations represent the number of discriminations that would be expected to occur by chance.

was done across the 4 stimuli and 2 trials of each stimulus for the 19 participants, with the probability of correct selection by chance on each trial of the 1 different SDC being 0.2. A statistically significant outcome permitted binomial tests across the 3 fatty acids and 2 trials of each for the 19 participants and for each fatty acid separately. In addition, binomial tests were done for the number of discriminations (selection of the 1 different SDC on both trials) across the 3 fatty acids for the 19 participants and for each fatty acid separately, with the probability of correct selection by chance on a pair of trials of the 1 different SDC being 0.04. Bonferroni corrections of P values were done (Table 2).

Results

Descriptive statistics

For the vapor-phase OCO presentation that this study used, 26% of participants (5 participants) discriminated oleic acid, 16% of participants (3 participants) discriminated linoleic acid, and 0% discriminated stearic acid (correct selection on both trials) (Figure 1). However, 84% of the participants (16 participants) discriminated vapor-phase OCO peppermint

Table 2 Binomial test outcomes: samples tested, testing parameters, and *P* values for correct selection on individuals trials and for discriminations[a]

Sample[b]	Number of trials	Number of correct selections	Chance probability	Binomial test *P* value	Bonferroni-corrected *P* value
Correct selections					
Four stimuli	152	74	0.2	**2.959×10^{-15}**	—
Three fatty acids	114	39	0.2	**0.0003**	—
Oleic acid	38	18	0.2	0.000136	**0.0004**
Linoleic acid	38	12	0.2	0.0623	0.125
Stearic acid	38	9	0.2	0.345	0.345
Discriminations[a]					
Three fatty acids	57	8	0.04	**0.0019**	—
Oleic acid	19	5	0.04	0.000743	**0.002**
Linoleic acid	19	3	0.04	0.0384	0.077
Stearic acid	19	0	0.04	1.0	1.0

P values ≤ 0.05 are in bold.
[a]Correct selection of the 1 different SDC on both trials.
[b]There were 19 participants. Each stimulus was presented twice.

extract, selecting the correct SDC on both of the available presentations (Figure 1 and Table 1). The median number of correct selections of the 1 different SDC by a participant was 1 for oleic acid, 0 for linoleic and stearic acids, and 2 (the maximum possible) for peppermint extract (Table 1).

Binomial tests

Across all 4 stimuli, and across the 3 fatty acids, there were 1 or more statistically significant differences from chance selections of the 1 different SDC ($P \leq 0.0003$). Subsequent binomial test analyses of the number of correct selections of the 1 different SDC for oleic acid indicated a statistically significant difference from chance ($P = 0.0004$). However, neither the number of correct selections of the 1 different SDC for linoleic acid ($P = 0.125$) nor the number of correct selections of the 1 different SDC for stearic acid ($P = 0.345$) was significantly different from chance, Bonferroni corrected (Table 2).

Binomial test analyses of the number of discriminations (selection of the 1 different SDC on both trials) were done across the 3 fatty acids and for each fatty acid. Across the 3 fatty acids, the number of discriminations was significantly different from chance ($P = 0.0019$). For individual fatty acids, the number of discriminations was significantly different from chance for oleic acid ($P = 0.002$) but did not reach statistical significance for linoleic acid ($P = 0.077$) or stearic acid ($P = 1.0$) (Table 2).

Discussion

Oleic acid

Across participants, correct selections of the 1 SDC containing oleic fatty acid occurred in 47% of trials, and discriminations of oleic acid were done in 26% of pairs of trials, with a median of 1 correct identification across participants of a possible 2 correct identifications. Both the oleic acid correct selections and the oleic acid discriminations differed significantly from chance ($P \leq 0.002$). It seems clear that vapor-phase oleic fatty acid was discriminated from a blank OCO. Nonetheless, correct selections of vapor-phase oleic fatty acid were appreciably less than the correct selections for peppermint extract, which occurred in 92% of trials, with discriminations on 84% of pairs and a median of 2 correct selection of peppermint extract.

The observed oral cavity discrimination of oleic acid fails to confirm the hypothesis that vapor-phase 18-carbon long-chain fatty acids cannot be discriminated OCO. That hypothesis was based upon a previous study (Bolton and Halpern 2010) that had not found statistically significant OCO discrimination of vapor-phase oleic, linoleic, or stearic fatty acids. The present study was done to explore the possibility that fatty acid concentrations higher than those employed by Bolton and Halpern (2010) might be discriminated OCO. Using a concentration approximately twice that employed by Bolton and Halpern (2010), this possibility was confirmed for vapor-phase oleic acid in the present study.

Linoleic acid

In the present study, linoleic acid was also used at approximately twice the concentration employed by Bolton and Halpern (2010), but the number of correct OCO selections was less than those for oleic acid, and a median of 0 correct selection of linoleic acid per participant was found of a possible 2 correct selections. Neither the

number of correct selections of linoleic acid nor the number of discriminations differed significantly from chance ($P \geq 0.077$).

The undiluted linoleic acid employed in the present study, provided at 81%, was at approximately twice the concentration that had been sufficient for orthonasal and retronasal discriminations of vapor-phase linoleic acid from blanks as well as retronasal discriminations of linoleic acid from other fatty acids (Bolton and Halpern 2010; Kallas and Halpern 2011). However, it could be suggested that if a more concentrated linoleic acid liquid had been used in the present study, perhaps sufficiently frequent OCO selection of the 1 SDC containing linoleic acid would have occurred to yield a nonchance outcome. Because higher concentrations of linoleic acid are available (see Linoleic acid ≥ 93%), the possibility of vapor-phase OCO detection of more concentrated linoleic acid cannot be dismissed.

Stearic acid

Stearic acid, presented OCO in vapor phase, received the lowest total number of correct selections (24% of trials; chance expectation would be 20%) and no discriminations (i.e., a total absence of correct selections on both presentations). Neither the number of correct selections of stearic acid nor the number of discriminations differed significantly from chance ($P \geq 0.345$). It might be suggested that a larger sample size might yield statistically significant discrimination results for vapor-phase OCO stearic acid. However, if the hypothesized larger sample size continued to present zero discriminations for stearic acid, then no matter how large the sample size were supposed, the probability of a difference from chance would remain constant at 1.0.

The stearic acid that was used in the present study had a purity of ≥95%, as was the case for Bolton and Halpern (2010) and Kallas and Halpern (2011). This precluded any substantial increase in concentration in the present study. However, because the stearic acid was not a liquid, its efficacy as a vapor-phase stimulus might be questioned. In a previous study that used the same amount and purity-level of stearic acid, inhaled in vapor phase from SDCs comparable to those of the present study, but instead presented orthonasally and retronasally (Bolton and Halpern 2010), the results were very different from the present OCO study. Bolton and Halpern (2010) reported that the SDC with stearic acid was selected in 83% of orthonasal trials and in 93% of retronasal trials, which exceeded the percentages of linoleic and oleic acids retronasal correct selections. As in the present study, the Bolton and Halpern (2010) experiment also had a Kimwipe square taped around the straw end inside the SDC (abbreviated as SPC in that study) to prevent possible particulate inhalation of stearic acid (or NaCl). It seems that the absence of OCO discrimination of vapor-phase stearic acid in the present study was not due to a lack of adequate stimulus presentation but instead to an absence of sufficient responsiveness by the oral cavity trigeminal sensory system.

Possible effects of the stimulus presentation sequence

Because the peppermint extract trials were always accomplished first in order to not continue with participants who might have a generally low sensitivity to vapor-phase stimuli, it is possible that those participants who continued in the study (19 of the 20 who began) expected a difference among the subsequent pairs of 5 SDC presentations as great as that between peppermint extract and its blank. This could conceivably have influenced their subsequent selections when fatty acids and their blanks were presented. Any possible effects of such expectations were not sufficient to suppress statistically significant selection and discrimination of oleic acid. In addition, it could be possible that the testing sequence, in which the peppermint extract was tested first, might have altered participants' sensitivity to subsequent stimuli. If any desensitization did occur, the next stimulus to be tested, linoleic acid, might have been especially affected. Both the possibility of heightened expectations and the posited desensitization might suggest that the present failure to observed a Bonferroni-correct P value ≤ 0.05 for vapor-phase OCO linoleic may not unequivocally demonstrate an absence of responses. Linoleic acid responses, per se, are considered further below (Bonferroni corrections).

Bonferroni corrections

One aspect of the appropriate level of confidence in the present study's analysis is the effect of Bonferroni corrections. Arguments have been made suggesting that Bonferroni corrections are too conservative (Perneger 1998; Nakagawa 2004), at least under some circumstances, although also contending that such corrections may be necessary (e.g., Bland 2000). For the present study, the P values for stearic acid, being the largest of the binomial test P values for the 3 fatty acids, received a Bonferroni correction of 1.0 (Table 2), and therefore would be unchanged ($\gg 0.05$) if the Bonferroni corrections were removed. The P values for oleic acid, which were appreciably less than 0.05 with Bonferroni corrections, would become even smaller without Bonferroni corrections.

However, for OCO vapor-phase linoleic acid discriminations (correct selection in both presented trials), removal of the Bonferroni correction would result in a change from a nonsignificant to a statistically significant outcome, with the uncorrected $P = 0.0384$ (Table 2). A possible desensitization of OCO linoleic acid responses in the present study due to the preceding peppermint trials has been noted, as has the possible effect of utilizing a higher concentration of linoleic acid. Overall, it is tempting to suggest that vapor-phase linoleic acid may be an OCO 18-carbon fatty acid stimulus, in addition to the vapor-phase oleic acid observed in the present study.

Why no oral cavity stearic acid discrimination?

The present data indicate that the oral cavity trigeminal sensory system responds to vapor-phase presentations of oleic

fatty acid, and perhaps to linoleic fatty acid, but not to vapor-phase stearic acid. This suggests that 1 and possibly 2 of the tested long-chain 18-carbon fatty acids can be sensed in the oral cavity before they reach the nasal cavities.

Because oleic, linoleic, and stearic acids are all 18-carbon fatty acids, possible explanations for the differences in OCO responses to these vapor-phase stimuli would be helpful. Oleic and linoleic acids are both unsaturated (having double bonds) fatty acids. Oleic acid is "monounsaturated," whereas linoleic is "polyunsaturated" (specifically, 2 double bonds). In more detail, both oleic acid and linoleic fatty acids have "cis" 3D configurations, resulting in the systematic ("scientific") names of cis-9-octadecenoic acid for oleic acid and cis-9, cis-12-octadecadienoic acid for linoleic acid (Zamora 2011). In contrast, stearic fatty acid is saturated, that is, no double bonds, and has the systematic name octadecanoic acid. Thus, although oleic, linoleic, and stearic acids are all 18-carbon fatty acids, there are substantial differences in their chemical structures, with stearic acid's straight-chain structure differing from both oleic and linoleic acids (see Leray 2011). Interactions between these different structures and the membrane receptors of oral cavity trigeminal sensory neurons presumably underlie the observed divergent responses.

In contrast to the observed absence of OCO responses to vapor-phase stearic acid, orthonasal presentations of vapor-phase stearic acid had produced correct selections of the 1 different SDC in 83% of orthonasal trials and in 93% of retronasal trials (Bolton and Halpern 2010). These levels of retronasal responses to vapor-phase stearic acid was comparable to that of the peppermint extract in the present OCO study. Furthermore, retronasally presented vapor-phase stearic acid was discriminated from retronasal vapor-phase linoleic acid in 83% of trials and from vapor-phase oleic acid in 75% of trials (Kallas and Halpern 2011). It is clear that the form of stearic acid utilized in the present study provided effective vapor-phase stimulation when access to the nasal cavities was available. It appears that restriction to the oral cavity, and the consequent limitation to the oral cavity trigeminal system, results in an absence of responses to the present vapor-phase stearic acid.

Oral responses to 18-carbon long-chain fatty acids

Within the oral cavity, 18-carbon long-chain fatty acids could be mechanical and/or chemosensory stimuli. In liquid phase, these fatty acids have been considered an important factor in the texture of foods (e.g., Drewnowski 1987, 1997). Human gustatory and/or somesthetic responses to liquid phase, 18-carbon long-chain fatty acids have also been reported (Chalè-Rush et al. 2007a, 2007b; Mattes 2009a, 2009b). In the latter studies, the fatty acids were presented in an emulsion, and responses were compared with an emulsion lacking fatty acids, thus controlling for effects of mechanical stimulation. In order to prevent movement of vapor-phase components to the nasal cavities, in those studies, a nose clip was used. These procedures served to minimize differential mechanical responses to the liquid fatty acids as well as to prevent nasal cavity responses to vapor-phase components as bases for the observed discriminations between oleic, linoleic, and stearic acids versus controls. However, for those studies, inside the oral cavity trigeminally based responses to vapor-phase components of the long-chain fatty acids could conceivably have occurred. The present study tested this possibility, finding positive results for oleic acid and suggestive results for linoleic acid, but no evidence for oral cavity responses to vapor-phase stearic acid. It follows that responses to some vapor-phase long-chain 18-carbon fatty acids can start in the oral cavity, before vapor-phase odorants reach the nasal cavities via a retronasal route.

In conclusion, the long-chain 18-carbon fatty acid oleic acid was discriminated from blanks when presented OCO in vapor phase. Under the same testing and analysis conditions, discrimination of another long-chain 18-carbon fatty acid, linoleic acid, did not reach statistical significance, but if a less conservative statistical approach was adopted, linoleic acid was discriminated at a nonchance level. A third 18-carbon fatty acid, stearic acid, failed to reach statistically significant levels of discrimination even if a less conservative statistical approach was adopted. These 3 fatty acids differ in structure, with the saturated stearic acid distinct from the unsaturated oleic and linoleic acids. The OCO discriminations of oleic acid, and possible discriminations of linoleic acid, indicate that one or perhaps both of these fatty acids, in vapor phase, may be responded to in the oral cavity before vapor-phase components reach the nasal cavities.

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