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Evidence that sensitivity to 6-n-propylthiouracil (PROP) affects perception of retro-nasal aroma intensity

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Abstract

6-n-propylthiouracil (PROP) is widely used for bitterness sensitivity tests, and individuals can be classified as being non-tasters (NT), medium-tasters (MT) or super-tasters (ST), with the last group giving the highest ratings to PROP. These perceptual differences can be very pronounced and extend to other compounds and taste modalities. We investigated whether this differential taste sensitivity between individuals extends to perception of aroma. Using a balanced, replicated design, 46 subjects (NT=15, MT=16, ST=15) used the Labeled Magnitude Scale to rate the intensity of aqueous solutions containing one of 3 odorants (acetaldehyde, diacetyl, and linalool) presented at 3 concentrations under 4 conditions: (C1) ortho-nasally, (C2) retro-nasally, (C3) retro-nasally with the addition of an astringent stimulant (aluminium sulfate) and (C4) retro-nasally with the addition of a bitter stimulant ((-)-epicatechin). Unexpectedly, ST rated odor intensities higher than NT and MT in C2, C3 and C4. Taken overall, the data suggest a 'global' advantage for ST with respect to perception of olfactory stimuli.

Key words: Food, health, PROP, 6-n-propylthiouracil, psychophysics, olfaction, flavor, taste, astringency, bitterness, aroma, supertasters.

Introduction

Genetic variation in perception of taste and tactile sensation: The ability to taste 6-n-propylthiouracil (PROP) and certain other bitter compounds is genetically inherited and has been thought to follow an incomplete dominance mode of inheritance³², allowing for the separation of individuals in a population into three distinct taster groups: non-tasters (NT), medium tasters (MT) and super-tasters (ST)³. Recent research into the molecular basis for PROP sensitivity has suggested that while low concentrations of PROP are detected by people with certain alleles of the bitter receptor gene TAS2R38, perceived intensity of PROP at high concentrations may not be strongly related to genotype^{9, 23} raising some doubts about the classification of individuals into three distinct taster groups.

ST experience PROP as intensely bitter, MT perceive PROP but less intensely than ST, and NT cannot taste PROP or experience it as a very mild sensation. These perceptual differences based on PROP taster status (PTS) extend to other bitterants^{2, 4, 6, 7, 17}; sweet compounds²⁹, salty compounds⁵ and substances that produce oral irritation/pain^{14, 35} and tactile sensations^{22, 66}, including astringency⁵⁰, although some studies have found no such associations with PROP sensitivity⁵⁹. PTS has also been associated with individual food preferences and selection^{1, 3, 20, 68}, body mass²¹, cardiovascular disease risk²⁴ and some forms of alcoholism¹⁸.

Physiologically, papillae and taste bud densities are positively correlated with PROP sensitivity, along with greater peripheral innervation of these structures^{44, 55}. Another genetic basis for

differences in taste perception has recently been reported. Higher taste intensity ratings were recorded for a variety of stimuli in individuals who perceive taste from thermal stimulation alone ('thermal taste')³¹. Interestingly, the authors also found that thermal tasters give higher intensity ratings to the odour of vanillin when presented both ortho-nasally and (particularly) retro-nasally, and suggest that differences in CNS processes involved in both taste and flavor are implicated.

Olfaction, flavour and cross-modality interactions: Suppression and enhancement effects amongst the different taste modes have been well-documented^{8, 40, 42, 43, 49, 58}. Numerous interactions between taste and aroma have also been reported, due to physical, physiological, cognitive or other psychological effects⁴⁷. For instance, taste may enhance aroma intensity⁴¹ or suppress it⁷⁰. Conversely, aroma may enhance²⁶ or suppress⁶³ taste intensity. Mediating factors in the direction and extent of these interactions include prior association with and similarity of the particular flavor-taste combination^{26, 27, 61, 62, 63} and whether a holistic or analytical paradigm is used in assessing the stimulant combinations^{28, 51, 52}. In addition, these cross modality interactions can be tastant and odorant specific^{26, 57}.

There is also evidence of cross-modality summation of sub-threshold taste and aroma compounds, demonstrating central neural integration of these sensations¹⁵. Irritants have also been shown to interact with both taste and olfaction, for instance suppressing sweetness⁵³ and some odors¹¹. Recent studies have

confirmed that tactile stimuli can also modify taste and aroma perception¹⁶. While the role of texture in controlling flavor release and thus aroma and taste intensity is significant and well documented^{37, 38, 48, 64, 71}, there is recent evidence that a perceptual integration of these modalities may also be involved^{12, 72}.

The common confusion between the words “taste” and “flavor”⁴⁶, the localization of flavor to the mouth⁴⁵ and the accrediting of taste qualities to odors (e.g., “That smells sweet!”)¹⁰ is further evidence of a general inability to perceive taste and odor independently⁵². Indeed, convergence among two or more of olfactory, taste or trigeminal stimuli is required to perceive the complex sensation of flavor¹⁶, with the orbitofrontal cortex representing a possible first cortical area of convergence for these three modalities, at least in primates⁵⁶.

PROP taster status and olfactory performance: The possible influence of PTS on ortho- or retro-nasal olfaction has not been rigorously investigated, perhaps a little surprisingly given the convergence phenomena outlined above. For instance, as ST experience a range of oral stimulants more intensely, it is possible that they may also perceive aroma more intensely due to integrative CNS mechanisms responsible for the processing of taste and olfactory signals. In a limited study investigating fat perception and PTS, NT had a significantly higher just-noticeable-difference olfactory threshold for diacetyl than either T or ST, although no difference was found for phenylethyl methyl ethyl carbamide, the other aromatic compound tested⁷³.

The current study sought to further determine the relationship between PTS and perceived aroma intensity as assessed both ortho-nasally and retro-nasally. Three beverage/food-relevant odorants were selected that differ in their aroma quality and chemical species (acetaldehyde, diacetyl and linalool). These odorants were presented at three different concentrations under four experimental conditions: (i) ortho-nasal perception in the absence of an oral stimulant (ii) retro-nasal perception in the absence of an oral stimulant (iii) retro-nasal perception in the presence of an astringent (aluminium sulfate) and (iv) retro-nasal perception in the presence of a bitterant ((-)-epicatechin). If differential sensitivity to perception of odorant intensity within the three PTS groups exists, then any differences in responses between conditions should help elucidate the relative involvement of peripheral vs. CNS processes.

Methods

Participants and PTS classification: Subjects were recruited from the Brock University student, staff and faculty populations and from wine enthusiasts who had indicated an interest in the project. Prospective participants were screened using a general health questionnaire, and individuals indicating gustatory or olfactory dysfunction were excluded from the study. A preliminary assessment of PTS was made using PROP-impregnated filter sticks⁵⁰, and a sub-set of these subjects was invited to participate further in the experiment. PTS was then more precisely determined using the one-test solution method⁶⁵, comprising of a 0.32 mM solution of PROP. The criteria for determining PTS were based on Tepper *et al.*⁶⁵, except that the adjective anchors on the labeled magnitude scale (LMS)³⁰ were used to separate taster groups instead of numerical distances from origin due to slight variations in the length of the computer-generated scale (Compusense™)

compared to that employed by Tepper *et al.*⁶⁵. Thus, non-tasters were designated as those who rated the solution as “moderate” or less (≤ 16.22 mm), medium-tasters gave intensity ratings between “moderate” and “very strong” ($16.22 \text{ mm} < x < 50.12 \text{ mm}$), and super-tasters indicated intensities corresponding to “very strong” or greater (≥ 50.12 mm). Means and standard deviations for PROP intensity ratings for NT, MT and ST were, respectively, 6.38 ± 4.55 ; 34.49 ± 11.33 ; 70.66 ± 16.51 .

A total of 46 participants completed the study; 39 were undergraduate or graduate students of Brock University and four were wine-enthusiasts or amateur wine-makers. Fifteen were designated as NT (9 females, 6 males), 16 were MT (10 females, 6 males) and 15 were ST (11 females, 4 males). The age of participants ranged from 18 to 55. Participation was voluntary, although entry into a draw for a small monetary prize was offered as an incentive.

Oral and olfactory stimuli: Acetaldehyde, diacetyl and linalool were all sourced from FCC, Aldrich Chemical Company, Inc, Milwaukee, WI. Bench-trials were conducted with ST, MT and NT not formally part of the study to find three perceptually different concentrations of each of these compounds (in aqueous solution) while still representing aroma intensities comparable to those found during everyday eating and drinking activities. Bench-trial participants were also asked to indicate the presence of any taste, astringent or other oral sensation elicited by the odorant solutions; no oral sensations were reported. Each of the final concentrations was designated as ‘low’, ‘medium’ or ‘high’ and are, respectively: acetaldehyde, 0.02, 0.2 and 2 g/L; diacetyl, 0.01, 0.1 and 1 g/L; linalool, 0.001, 0.01 and 0.1 g/L (all in aqueous solution using distilled water). A few drops of 95% ethanol (LCBO, Ontario, Canada) were required to solubilize the linalool.

The astringent aluminium sulfate (Fisher Chemicals Scientific, New Jersey) and the bitterant (-)-epicatechin (Aldrich Chemical Company, Inc, Milwaukee, WI) were selected as oral stimulants and bench tested for concentration (in aqueous solution) by ST, MT and NT not formally part of the study. The eventual concentrations selected gave separation of intensity ratings between the different PTS groups used for bench testing (data not shown), and were aluminium sulfate 0.25 g/L and (-)-epicatechin 0.4 g/L (both in aqueous solution using distilled water). These were the final concentrations used in the solutions presented in Conditions 3 and 4, respectively (below). A 5 g/L pectin solution was prepared using pectin (Copenhagen Pectin Genu, Quest International Canada, Inc., Lachine, Quebec) and boiling water, and after cooling was used by participants as a mouth rinse between oral stimuli to cleanse the palate and reduce possible carry-over effects.

Assessment of weights for standardization: In order to standardize for possible idiosyncratic use of the LMS among different PTS groups, three weights of mass 225 g, 558 g and 999 g were constructed by filling Rubbermaid™ drinking bottles with salt and/or sugar and wrapping them with aluminum foil (adapted from Delwiche *et al.*¹⁷). Each weight was presented with a three-digit blinding code and in order of ascending mass. Participants assessed them for ‘heaviness’ in duplicate using a LMS in two separate sessions. Full details on the preparation of all stimuli can be found elsewhere³³.

Experimental design and conditions: The general experimental plan involved four conditions:

Condition 1 (Sessions 1 & 2): Evaluation of ortho-nasal aroma intensity elicited by aqueous solutions of acetaldehyde, diacetyl and linalool at low, medium and high concentrations

Condition 2 (Sessions 1 & 2): Evaluation of retro-nasal aroma intensity elicited by stimuli in Condition 1 (C1)

Condition 3 (Sessions 3 & 4): Evaluation of retro-nasal aroma intensity elicited by stimuli in C1 with the addition of the astringent aluminum sulfate to the solutions

Condition 4 (Sessions 5 & 6): Evaluation of retro-nasal aroma intensity elicited by stimuli in C1 with the addition of the bitterant (-)-epicatechin to the solutions

The concentrations of the compounds used are given above. Evaluations took place in individual booths under red lighting (130 volt, 100 watt Haskellite red bulb with red cellophane over top) within the specialized sensory evaluation laboratory in the Cool Climate Oenology and Viticulture Institute at Brock University. Participants assessed nine different stimuli (three sets of three samples) in each of six one-hour sessions. Duplicate evaluations of each stimulant were made and a randomized block design was used. The presentation order for each of the three concentrations of each odorant was also randomized. 20 mL samples of each solution were poured 30 minutes before evaluation, presented in ISO glasses covered with a Petri dish cover, and identified with a 3-digit random number. After verbal and computer instruction at the start of each session, participants assessed samples for ortho-nasal or retro-nasal aroma intensity using a computer-generated LMS (Compusense® *five* release 4.4, Compusense Inc., Guelph, ON, Canada).

Sample assessment protocols for each condition were standardized. Ortho-nasal evaluation consisted of three short sniffs of each sample prior to rating of intensity. A minimum 1 min break between samples within a set and a 3-10 min break between sets was enforced. Retro-nasal assessment required participants to use a nose plug at the start of each evaluation to eliminate ortho-nasal stimulation. The entire sample was swirled around the mouth for 5-10 secs before being expectorated. Participants were then instructed to breathe in through the mouth, remove the nose plug, close the mouth and breathe out through the nose. After exhaling, retro-nasal aroma intensity was immediately rated on the LMS. For Conditions 3 and 4, a second LMS was then completed for intensity of astringency and overall in-mouth sensation, respectively. Finally, degree of 'liking' for each sample and condition was assessed using a 9-point hedonic scale ¹⁹.

A minimum 1.5 min break between samples within a set and a 3 min break between sets was enforced for Condition 2. For Conditions 3 and 4, the break was 5 min between samples and 10 mins between sample sets. Also for these conditions, the pectin mouth rinse solution was used between samples, followed by double rinsing with water ¹³. Water and unsalted crackers were also available as required for all conditions to further assist in minimizing carry-over effects and palate fatigue ⁶⁷. All panelists participated in at least one 'practice' run using the evaluation protocols before data collection began.

Data analysis: A correlation analysis (Pearson) was performed on the average intensity scores for the duplicate assessments of each of the three weights and correlated with the individual

intensity ratings of the 0.32 mM solution of PROP for each participant. For the 225 g, 558 g and 999 g weights, the r-values obtained were 0.063 (p=0.679), 0.124 (p=0.412) and 0.124 (p=0.413), respectively. Therefore, raw data from the ortho-nasal and retro-nasal trials were not standardized.

Statistical analyses of data from Conditions 1-4 were conducted using SPSS 11.5 software for Windows (SPSS Inc., Chicago, IL, USA). A mixed-model 3x3x3x2 analysis of variance (ANOVA) was used to test for between and within-subject effects (between subjects variable: PTS; within subjects variables: odorants, concentrations, replication). Mauchly's test of sphericity was conducted on the within-subject effects. If the sphericity assumption was violated, the Huynh-Feldt correction factor was used to correct the degrees of freedom ³⁶. Bonferroni_{0.05} was used as the means separation test.

An *a priori* decision was made to use orthogonal contrasts to compare the responses across PTS groups. Also, in recognition of the difficulties in accurately categorizing MTs and in order to improve statistical power ⁶⁹, we collapsed the data from the NT and MT groups. Thus, mean responses for ST were compared to the combined mean for the NT and MT groups. Only differences corresponding to p<0.05 are reported as statistically significant.

Results

Ortho-nasal (Condition 1)

Major finding: As expected, no main effect of PTS was found for the ortho-nasal evaluation across odorant, concentration and replication (F=2.151, df=2.43, p=0.129). Means and standard errors for NT, MT and ST were, respectively, 25.92 ± 2.92, 23.53 ± 2.82 and 31.75 ± 2.92. Contrast analysis showed no statistical difference between the means of ST and the means of the combined NT+MT group at an alpha of 0.05, although the results approach significance (contrast estimate=-7.06, p=0.051).

Additional findings: Across PTS groups, there was a significant main effect for replication (F=4.32, df=1.43, p=0.044), odorant concentration (F=77.52, df=1.38, p=0.00) and for odorant * concentration (F=7.27, df=3.58, p<0.001). The mean aroma intensity score for replicate 1 was lower than replicate 2; possibly indicating increased familiarity and therefore confidence in the use of the scales by participants. The concentration effect was expected; the intensity scores for each of the three concentrations were significantly different from each other for each odorant (Bonferroni_{0.05}). The mean score ± standard error for each odorant for low, medium and high concentrations respectively, were: acetaldehyde 19.92±1.56, 26.53±1.94, 36.22±2.66; diacetyl 22.29±2.20, 25.51±2.44, 37.96±2.96; linalool 13.27±1.56, 25.15±1.74, 36.76±2.22. There was no difference in the mean hedonic scores between the three PTS groups (F=0.26, df=2.43, p=0.770), although across PTS groups some differences were found for odorant, concentration and odorant*concentration (data not shown).

Retro-nasal (Condition 2)

Major finding: Contrary to expectation, there was a significant PTS effect for retro-nasal aroma intensity elicited by the odorant solutions in the absence of other oral stimulation (F=4.46, df=2.43, p=0.017). Averaged across odorant, concentration and replication, ST rated aroma intensity higher than MT, while there were no differences between NT and either of the other 2 PTS groups

(Table 1). Contrast analysis revealed that the mean score of the combined NT+MT group was significantly lower than that for ST (estimate=-8.707, p=0.006).

Additional findings: There were significant within-subject effects for concentration (F=92.67, df=1.56, p<0.001) and odor * concentration (F=12.71, df=3.73, p<0.001). The concentrations of each of the three odorants had been selected to elicit differences in retro-nasal aroma intensity; the mean score ± standard error for each for low, medium and high concentrations respectively, were: acetaldehyde 11.44±1.30, 21.07±1.85, 30.89±2.51; diacetyl 13.04±1.67, 18.63±1.69, 24.87±2.19; linalool 10.13±1.15, 16.14±1.63, 35.98±2.72. There was no significant difference in the mean hedonic scores between the three PTS groups (F=0.23, df=1.43, p=0.796), although differences were found for odorant, concentration, and replicate * odorant * PTS (data not shown).

Retro-nasal + astringent (Condition 3)

Major finding: There was a significant PTS effect for retro-nasal aroma intensity elicited by the odorant solutions in the presence of aluminium sulfate (F=3.48, df=1.42, p=0.040). Averaged across odorant, concentration and replication, ST rated aroma intensity higher than both NT and MT, while there were no differences between the scores of NT and MT (Table 1). Contrast analysis showed that the mean score of the combined NT+MT group was significantly lower than that for ST (estimate=-9.547, p=0.011).

Additional findings: There were significant within-subject effects for odorant (F=3.88, df=2.84, p=0.025), concentration (F=111.45, df=2.84, p<0.000), odorant * concentration (F=21.34, df=3.10, p<0.000), concentration * PTS (F=2.62, df=4.84, p=0.041), replicate * odorant * PTS (F=2.65, df=4.84, p=0.039) and replicate * odorant * concentration (F=2.73, df=4.17, p=0.031). The mean score ±

Table 1. Mean retro-nasal aroma intensity ratings¹ of non-tasters (n=15), medium-tasters (n=16) and super-tasters (n=15) elicited by odorant solutions² with and without concurrent oral stimuli.

Stimuli	PROP taster status group		
	Non-tasters	Medium-tasters	Super-tasters
Odorant (Condition 2)	18.77 ± 2.49 ab	15.94 ± 2.41 b	26.02 ± 2.49 a
Odorant + astringent ³ (Condition 3)	20.21 ± 3.07 a	21.25 ± 2.87 a	30.31 ± 2.97 b
Odorant + bitterant ⁴ (Condition 4)	19.78 ± 2.82	18.30 ± 2.73	26.35 ± 2.82

¹Data shown are mean values ± std errors and averaged across 3 odorants, 3 concentrations and duplicate assessments. Means with different letters within each stimulus condition are significantly different (Bonferroni_{0.05} after significant F-value from ANOVA, α=0.05); ²Odorant solutions consisted of aqueous preparations of one of the following: acetaldehyde, 0.02, 0.2 and 2 g/L; diacetyl, 0.01, 0.1 and 1 g/L; linalool, 0.001, 0.01 and 0.1 g/L; ³aluminium sulfate 0.25 g/L; ⁴(-)-epicatechin 0.4 g/L.

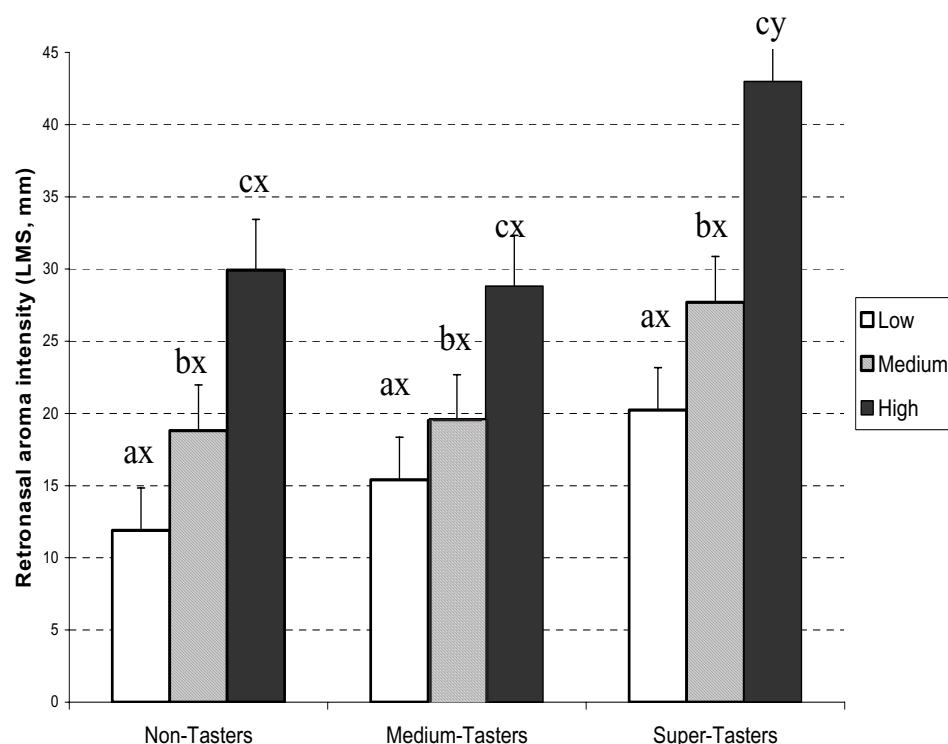


Figure 1. Mean retro-nasal aroma intensity ratings of non-tasters (n=15), medium-tasters (n=16) and super-tasters (n=15) elicited by solutions containing odorants at 3 concentrations (low, medium, high) plus aluminium sulfate. (Data are mean values averaged across 3 odorants and duplicate assessments. Odorant identity and concentrations given in text. Within each PTS group, concentration means sharing the same letter (abc) do not differ; within each concentration, means sharing the same letter (xyz), do not differ [Bonferroni_{0.05}]).

standard error for each odorant for low, medium and high concentrations respectively, were: acetaldehyde 16.20±2.02, 23.50±2.53, 32.75±2.56; diacetyl 18.07±2.04, 20.71±1.72, 27.05±1.92; linalool 13.27±1.59, 21.80±1.85, 41.95±2.78. The concentration * PTS result is particularly interesting. As Fig. 1 illustrates, as concentration of odorants increases, so does retro-nasal aroma intensity for all PTS groups. While a trend is suggested of higher ratings for ST compared with both NT and MT at low and medium concentrations, ST rate intensity significantly higher than the other two groups at high odorant concentrations.

Astringency: Astringency intensity data was also collected for this condition in order to minimize potential dumping effects on retro-nasal aroma intensity and to provide additional information on PTS and its relationship with astringent sensations. A significant main effect of PTS was found for astringency intensity ($F=5.14$, $df=1.42$, $p=0.010$). ST rated the intensity higher than both NT and MT, while NT and MT did not differ (Bonferroni_{0.05}). Means and standard errors were: NT, 25.56±3.36; MT, 30.36±3.14; ST, 40.18±3.25. Concentration ($F=15.56$, $df=1.70$, $p<0.0001$) and odorant * concentration ($F=2.76$, $df=4.17$, $p=0.030$) were significant within-subject effects (data not shown).

Retro-nasal + bitterant (Condition 4)

Major finding: Contrary to expectation, ANOVA did not show a PTS effect for retro-nasal aroma intensity elicited by the odorant solutions in the presence of the bitterant (-)-epicatechin ($F=2.36$, $df=2.43$, $p=0.107$; Table 1). However, using contrast analysis, ST showed a significantly higher mean response than that of the combined NT+MT group (estimate=-8.053, $p=0.046$).

Additional findings: Significant within-subject effects were observed for odorant ($F=7.51$, $df=2.86$, $p=0.001$), concentration ($F=123.69$, $df=1.55$, $p<0.001$) and odorant * concentration ($F=21.97$, $df=3.49$, $p<0.01$). The mean score ± standard error for each odorant for low, medium and high concentrations respectively, were: acetaldehyde 12.20±1.41, 18.70±1.42, 27.84±2.17; diacetyl 15.43±2.00, 20.49±1.98, 26.23±2.43; linalool 11.66±1.28, 20.68±1.92, 40.04±2.87.

Intensity data for *overall in-mouth sensation* was also collected for this condition to minimize potential dumping effects on retro-nasal aroma intensity. The term *overall in-mouth sensation* was selected because although (-)-epicatechin elicits primarily a bitter taste, an astringent side-sensation has also been reported⁶⁷. Unexpectedly, no PTS effect was found for intensity of *overall in-mouth sensation* using ANOVA, although the results approach statistical significance ($F=2.98$, $df=2.43$, $p=0.061$). However, ST had a higher mean response when compared to the combined NT+MT group (contrast analysis, estimate= -9.433, $p=0.018$). Mean intensity ratings and standard errors were: NT, 21.53±3.20; MT, 20.21±3.10; ST, 30.28±3.20. Odorant ($F=3.93$, $df=2.86$, $p=0.023$), concentration ($F=55.67$, $df=1.49$, $p<0.000$) and odorant * concentration ($F=11.03$, $df=3.34$, $p<0.000$) were also significant within-subject effects. Collapsed across replicates and PTS, a trend of increasing intensity ratings for *overall in-mouth sensation* with increasing odorant concentration is observed for all three odorants (data not shown).

Discussion

We hypothesized that any differential sensitivity to perception of odorant intensity across the three PTS groups would be limited to retro-nasal aroma, and only when an oral stimulus such as aluminium sulfate or (-)-epicatechin was presented concurrently. As we expected, ST gave significantly higher intensity ratings to retro-nasal aromas in the presence of oral stimuli (Conditions 3 and 4). However, contrary to our expectations, we found a similar pattern of results in Condition 2, where retro-nasal aromas were presented in the absence of oral stimuli. This pattern of results, combined with the relative robustness of the effect across diverse odorant groups and concentrations, suggests a global advantage for ST with respect to perception of olfactory stimuli that are presented retro-nasally. To determine the generality of these effects, further research is needed using other odorants and oral stimulants.

While overall, ST appear to be generally more sensitive than MT and NT to retro-nasal aromas, the presence of oral stimuli may also play a role in determining the degree of this sensitivity. The data for Condition 3 indicate that under certain conditions the presence of oral stimulants affects retro-nasal perceptions. Specifically, we found evidence that odorant intensity and the presence of an oral stimulus, in this case aluminium sulfate, may interact to further enhance the retro-nasal sensitivity of ST at higher odorant concentrations, suggesting that central neural integration must be occurring. The nature of cross-modality interactions has been found to be complex and dependent on the particular tastants and ortho-nasally presented odorants under investigation^{26,57}. It seems likely that complexity will also be found to characterize the interactions between tastants and retro-nasally presented odorants.

It is noteworthy that when odorant solutions were presented ortho-nasally (Condition 1), the pattern of differences in intensity responses across the three PTS groups was similar to that observed in the three retro-nasal conditions, but the results fell just short of statistical significance. Although panelists had received training before participating in Condition 1, their relative lack of familiarity with the evaluation protocols at this early stage of the experiment may have been a factor in the failure to detect a statistically significant effect. Other findings⁷³ also suggest that ortho-nasal aroma perception may be related to PTS, at least for some odorants, so further research is clearly in order to examine the nature and extent of differences across PTS groups with respect to ortho-nasal perception of olfactory stimuli.

Given the general agreement that PTS groups differ more in their perception of bitterness intensity than for other oral sensations, we expected significant separation between the groups for retro-nasal intensity in C4. However, the PTS effect in the presence of the bitterant (-)-epicatechin was weaker than expected. While it can be speculated that an alternative bitterant or a higher concentration of (-)-epicatechin may have yielded a larger effect for retro-nasal intensity of the odorants, the bitterness ratings were consistent with the magnitude of intensity responses reported in other PROP studies and typical of those experienced in real life ingestion of food and beverages⁵⁰. This finding further supports the aforementioned speculation that the enhanced perception of ST to aromatic stimuli is robust; that is, it is relatively independent of the quality of the concurrent oral stimulant/stimuli.

Not accounted for in this study were potential chemical and

physical interactions between the oral stimulants and the odorants. For instance, it is conceivable that changes in partition coefficients or solution viscosity from the addition of the oral stimulants may have altered the volatility of the odorants and thus perception of aroma intensity. While we speculate that any such changes would have had negligible effects on the suprathreshold aroma intensity ratings obtained here, further work could address this by conducting headspace analysis on samples using gas chromatography-based techniques.

Further considerations: The intensity responses of MT were very similar to those of NT for most olfactory and oral stimuli. This may be reflective of the actual distribution of intensity responses between the three PTS groups, particularly given the more complex stimuli used in this experiment in comparison with many psychophysical studies, but may equally be related to the widely reported difficulties in accurately and reliably categorizing PTS groups⁵⁴, particularly MT. Indeed, the validity of three distinct PROP taster groups as a proxy for genotype has been placed in some doubt with recent molecular studies^{9, 23}. Future research could consider adoption of genotype-based PTS classification methods or use of regression/correlation analysis (perceived intensity of olfactory stimuli vs. PROP intensity rating) to potentially address some of these concerns. Additionally, the number of subjects in each PTS group is relatively small in this study; replication of the trials with a larger n would increase the level of confidence in the conclusions drawn here.

The question of differential sensitivity to astringent sensations between PTS groups is equivocal in the literature, with studies failing to show an effect in red wine³⁴, with grape seed tannin^{47, 60} or with aluminium sulfate⁵⁰. However, in the former two studies, PTS categorization was based on subjects' threshold sensitivity to PROP, an approach that is considered by some as inferior to suprathreshold methods employing the LMS for the separation of PTS groups. Using this latter method, NT were shown to give lower intensity ratings for astringency elicited by red wine than MT and ST⁵⁰. The oral intensity data presented in this current study (C3) provide further evidence that perception of astringency intensity covaries with PROP sensitivity. The discrepancy with Pickering *et al.*⁵⁰ where no PTS effect was found for aluminium sulfate may be attributable to the use of a substantially lower concentration in the present study, and/or a mediating influence of olfaction from the presence of odorants in the current study.

Phenolic compounds such as (-)-epicatechin and tannins are important constituents of raw and processed vegetables and fruits (including wine and teas), and are of much interest given the general protective effect of this chemical group against cardiovascular, cerebrovascular and cancer-related diseases³⁹. Averaged across odorants and concentration, our data shows that ST experience the oral sensation(s) elicited by (-)-epicatechin more intensely than NT and MT. Greater taste acuity in ST for the bitter bioflavonoid naringin has previously been reported²⁰, although no PTS effect was found for bitterness response to grape seed tannin⁶⁰ or (-)-epicatechin¹⁷. It is possible that limitations from the use of threshold methods to classify individuals into PTS groups in the former study and low statistical power from only four subjects in two of the PTS groups in the latter underlie these null results.

Implications for food behavior: The ability of odorant type and concentration to affect the intensity of oral sensations, independent of PTS, has also been shown in this study and warrants further investigation. Of particular interest to the food and health industries will be opportunities to optimize the sensory properties of foods high in health-promoting phenolic compounds in order to influence preference and acceptance behaviors. This should involve consideration of cross—modality interactions, including those reported here.

Although somewhat equivocal in the literature, numerous studies have shown that ST differ in their liking of certain foods and in the foods they choose to consume because of their enhanced sensitivity to taste and tactile stimuli^{1, 3, 20, 25, 68}. The data presented here does not support the notion that differential liking in ST extends into the domain of olfaction; no PTS effect was found for hedonic responses to the odorants in either the ortho-nasal or retro-nasal modes. However, this finding may be of restricted application and not directly applicable to foods and beverages as the odorant solutions were not commercially realistic products with respect to composition, and no attempt was made to optimize their sensory appeal. Indeed, liking scores tended to be low for all participants; averaged across modes and stimuli, samples were rated at between *dislike slightly* and *neither like nor dislike* on the 9-point hedonic scale.

There is an obvious need for further elucidation of other physiological, social and cultural factors that may contribute to individual food preference and selection. We suggest consideration of food adventurousness⁶⁹, thermal taste sensitivity³¹ and their potential interaction with PTS as recent and particularly interesting areas for further investigation.

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