Chem. Senses 30: 9–21, 2005 doi:10.1093/chemse/bjh251

Do Tastants Have a Smell?

J. Mojet[1], E.P. Köster[2] and J.F. Prinz[3]

[1]Agrotechnology and Food Innovations, Wageningen University and Research, Wageningen, The Netherlands, [2]Department of Psychology, Utrecht University, Utrecht, The Netherlands and [3]TNO Nutrition and Food Research, Zeist, The Netherlands

Correspondence to be sent to: J. Mojet, Agrotechnology and Food Innovations, Bornsesteeg 59, 6700 AA Wageningen, The Netherlands. e-mail: jos.mojet@wur.nl

Abstract

The stimuli used in taste research are usually considered to be odourless. This was tested in two experiments with aqueous solutions of two representative compounds for each of the five taste qualities including umami. In the first experiment elderly and young subjects rated the intensity and pleasantness of three concentrations of the stimuli, while wearing or not wearing a noseclip. Saliva production was also measured. Blocking olfaction only influenced salivation for umami. It reduced taste intensity ratings, but as in an earlier experiment with the same compounds in food products, this effect was stronger in the young, who also liked the stimuli better wearing the noseclip. In the second experiment, another group of young people tried to detect the odours of the tastants dissolved in demineralized, double-distilled or Evian water. A considerable number of subjects could regularly detect seven of the ten tastants by olfaction and the extent to which they did correlated significantly with the reduction in taste intensity ratings for the different tastants found in the first experiment. We suggest that most tastants can be smelled and that this smell contributes to taste intensity ratings.

Key words: ageing, intensity, olfactory deprivation, pleasantness, saliva, taste

Introduction

The stimuli used in taste experiments are supposed to be non-odorous and to be perceived by the gustatory system only. Recently, some doubts about the veracity of this supposition have been raised. Mojet *et al*. (2003) compared the taste intensity perception of elderly and young people for ten (two per basic taste, including umami) taste stimuli. The young perceived the stimuli to be significantly more intense than did the elderly, but when a noseclip was worn, the young lost this advantage and judged the stimuli to be as intense as did the elderly under both nose clip conditions. Obviously, the young relied on other sensory information than taste alone when they judged the stimuli without a noseclip, whereas the elderly did not. Combined with the well-documented finding that olfactory sensitivity decreases more rapidly with age than gustatory sensitivity (Murphy *et al*., 1991), the authors hypothesized that olfaction was more involved in taste perception than is usually assumed. Two alternative explanations were proposed. The first suggested that weak odours, produced by the tastants themselves, could be smelled by the young, but not by the elderly. The other suggested that the presence of the tastants interacted with the olfactory perception of the medium and might intensify the odour of the products to a degree that could be noticed by the young, but not by the less-sensitive elderly. This latter possibility was plausible, since a number of the tastants (NaCl, KCl, MSG and IMP) are known flavour enhancers, but the fact that the same subjects showed a comparable age difference in sensitivity even when the stimuli were dissolved in distilled water and judged without a noseclip seemed to favour the first explanation. That the age effect for acetic acid, which is known to have an odour at higher concentrations, was among the strongest, pointed in the same direction.

Additive intensity perception effects between taste and smell have been reported in odour–taste mixture studies (Murphy *et al*., 1977; Murphy and Cain, 1980) and in odour–taste enhancement studies (Frank and Byram, 1988; Shaffer and Frank, 1990; Stevenson *et al*., 1999).

Retro-nasal smell was perceived by the subjects as taste from the oral cavity and thereby added to the taste intensity. Ortho-nasal smell, which has much weaker effects than retro-nasal smell (Zoeteman, 1978), does not have any effect on the intensity of simultaneously presented tastants (Hornung and Enns, 1984). Although in a number of these studies it was carefully verified that the odorants used in the mixtures were tasteless, none of the authors verified the lack of odour of the tastants. For odour-enhancement studies this seemed irrelevant, since

in comparing a tastant solution with that same tastant solution plus an odorant, the possible odorous properties of the tastant itself stayed constant. In the mixing studies, varying the concentration of the tastant and thereby adding small amounts of its odour may have influenced the total odour intensity considerably (Köster, 1968, 1969), thus leading to a much larger change in the total intensity impression than would be expected on the basis of the odour of the tastant alone.

Effects of the weak odour of the tastants may thus have played an important role in the differences between noseclip conditions in the study of Mojet *et al.* (2003), and the age-related loss of taste intensity found by them might be explained mainly by loss of olfactory sensitivity in the elderly.

Unfortunately, Mojet *et al.*'s (2003) experiment lacked a condition in which the tastants dissolved in water were judged while the subjects wore a noseclip. Here, it will be verified whether the difference in taste intensity perception between the elderly and young also disappears under olfactory deprivation when the tastants are dissolved in water.

In the first experiment a 'with noseclip' condition is compared with a 'no noseclip' condition for all ten tastants dissolved in water with new groups of elderly and young people. Apart from intensity, pleasantness and saliva production are also measured to check whether intensity differences between the two noseclip conditions are related to irritation about wearing a noseclip and to estimate the possible role of saliva production on intensity judgements.

Variations in salivary flow rates may explain individual differences in taste sensitivity (Christensen, 1986; Spielman, 1990; Guinard *et al.*, 1998; Neyraud *et al.*, 2003). Simple dilution by saliva, changing the pH by buffering acid tastants and altering the concentration of very dilute tastants by addition of salivary sodium are the mechanisms described. Although reports concerning the relationship between ageing, dry mouth syndrome and salivary flow rates are conflicting (Pederson *et al.*, 2002; Bradley and Beidler, 2003), taste intensity differences between age groups might also be explained by reduction in saliva production. Saliva production is taste quality dependent (Dawes and Watanabe, 1987) and increases with concentration and number of taste stimuli involved (Froehlich *et al.*, 1987; Watanabe and Dawes, 1988; Bardow *et al.*, 2001).

In a second experiment, the possibility of ortho-nasal olfactory stimulation by all ten tastants dissolved in water is directly studied in young subjects. To check whether odour effects found might be due to interactions of the tastants with possible water odours, each of these tastants is dissolved in three differently treated waters.

Experiment 1

Subjects

Nineteen older subjects (age 60–83 years: 10 male, mean age 69.0 years, SD 6.8; and 9 female, mean age 64.8 years, SD 2.9) and 20 young subjects (age 18–30 years: 10 male, mean age 23.5 years, SD 3.9; 10 female, mean age 21.3 years, SD 2.5) participated in the first experiment. All subjects were Caucasian and met the following criteria: healthy, not on a therapeutic diet, not living in a home for the elderly, not taking any prescribed medicine, non-smoking, no heavy alcohol users, non-pregnant or lactating, not subject to food allergies, good dental hygiene, and not wearing dentures (one subject had dentures but did not wear these while tasting the stimuli). Subjects were selected on a volunteer basis in response to advertisements in local newspapers and on bulletin boards in senior citizen centres. At the end of the experiments the subjects were paid for their participation. Of the 40 subjects that were recruited, one elderly subject was left out of the analyses since she clearly did not understand the task.

Stimuli

The saltiness, sweetness, sourness, bitterness and the perception of umami taste were investigated. In view of the purpose of the experiment, all stimuli were of the highest degree of purity available. They were presented in three suprathreshold concentrations (0.4 log step differences: see Table 1). The range of concentrations was similar to the range of concentrations used in a previous experiment (Mojet *et al.*, 2003). The compounds were dissolved in distilled water and stored below 4°C. They were presented on the following two days after acclimatization to room temperature. The subjects received 5 ml in a disposable 30 ml plastic cup with lid.

Procedure

Separate sessions were held for the elderly and the young for practical reasons, and each age group was split into two groups (see Table 2). Half of the elderly and half of the young subjects started to assess the stimuli in a session while wearing a noseclip and subsequently assessed them while not wearing a noseclip, the others did so in the reverse order. The sessions were held on two consecutive days.

All subjects received the taste qualities in the same fixed order per session. In the first series of session 1 and session 2, the order was sweet, sour, salty, bitter and umami, and in the second series the order was salty, sour, bitter, sweet and umami. Umami was always given last to avoid the risk that these compounds might cause an enhancement of the perception of a subsequent taste stimulus.

The order of the two tastants within one taste quality was held constant. For sweet the order was sucrose–aspartame; for salty, sodium chloride–potassium chloride; for sour, acetic acid–citric acid; for bitter, caffeine–quinine hydrochloride; and for umami, MSG–IMP. The three concentrations of each tastant were randomized per session series.

Summarizing, the subjects had to taste five taste qualities in a row, six stimuli per taste quality (three concentrations of compound A, and three concentrations of compound B). In addition, each series of one taste quality was preceded by a rinsing of the mouth with 5 ml distilled water, and

Table 1 Purities and concentrations of taste compounds

Compound*	Grade	Purity	Concentrations in g/l		
Sodium chloride (NaCl)	purissimum	>99.5 %	3.58	9.00	22.60
Potassium chloride (KCl)	purissimum	>99.8 %	5.68	14.26	35.83
Saccharose	purissimum	>99.5 %	8.55	21.48	53.95
Aspartame	reagent	>98.0 %	0.06	0.15	0.37
Acetic acid	purissimum	>99.8 %	0.63	1.59	4.00
Citric acid	reagent	>99.5 %	1.26	3.16	7.92
Caffeine	purissimum	>98.5 %	0.16	0.40	1.00
Quinine HCl	reagent	>99.0 %	1.29×10^{-3}	3.24×10^{-3}	8.13×10^{-3}
Monosodium Glutamate (MSG)	reagent	>99.0 %	1.99	5.01	12.58
Inosine '5-monophosphate (IMP)	reagent	>99.0 %	1.26	3.16	7.94

*All compounds supplied by Boom BV, Meppel, the Netherlands.

Table 2 Subject groups with their order of assessment

Group	1	2	3	4
age	elderly	young	elderly	young
female	5	5	5	5
male	5	5	5	5
day 1	noseclip off	noseclip off	noseclip on	noseclip on
	noseclip on	noseclip on	noseclip off	noseclip off
day 2	noseclip on	noseclip on	noseclip off	noseclip off
	noseclip off	noseclip off	noseclip on	noseclip on

was completed by a rinsing of the mouth with 10 ml distilled water. All stimuli, water and tastants, had to be swirled through the mouth for 5 s and to be spat out into the cups and covered carefully by the same lids. This time span of 5 s was chosen for two reasons. Firstly, because the saliva flow rate is highest at the beginning and then drops by the second to about half of the initial rate in ~11 s, due to a rapid adaptation. Secondly, to prevent fatigue on the part of the subject. The interstimulus interval of 45 s and the tasting time of 5 s were indicated by a tone signal. The tastants were rated on a nine-point intensity scale, ranging from very weak to very strong, and were also rated on a nine-point pleasantness scale, ranging from very unpleasant to very pleasant. All samples (both for the tastant and the water stimuli) were weighed immediately after each session to assess saliva production.

Statistical analysis

Methods

The statistical analyses were conducted by means of SAS®. Data were averaged arithmetically over the two replications. After checking for normal distribution, multivariate repeated-measures analysis was applied to investigate the effect of age and concentration on intensity perception, on liking and on saliva weight. The relationships between intensity, liking and saliva weight were investigated with correlation analysis. Furthermore, the effect of blocking the olfactory input on the intensity perception was compared for this experiment and for a previous one, which was carried out with the same tastants (Mojet *et al.*, 2003).

Levels of significance

All effects that have a *P*-value of 0.05 or lower are reported as 'significant'. Power analysis shows that, with the number of subjects in our study, an effect with a magnitude of 1.3 standard deviations and a *P*-value of 0.10 still has a power of 0.90. Therefore, a selection of the more interesting effects with a *P*-value between 0.05 and 0.10 are also reported. These effects will be denoted as 'trends'.

Results

Intensity perception

An overview of the results obtained under the 'without noseclip' and under the 'with noseclip' conditions is given in Figure 1 for young and elderly men and women separately. Before describing the effect of blocking olfactory input, which is the primary objective of this experiment, some general findings on the intensity perception of the tastants in water by young and older subjects will be briefly pointed out. Firstly, it is clear that the influence of the increase in concentration by 0.4 log concentration steps was different for the different taste qualities, and sometimes even for the different compounds within a taste quality. In some cases (NaCl and KCl; sucrose and aspartame), these differences can perhaps be ascribed to insufficient intensity matching and to the different position the

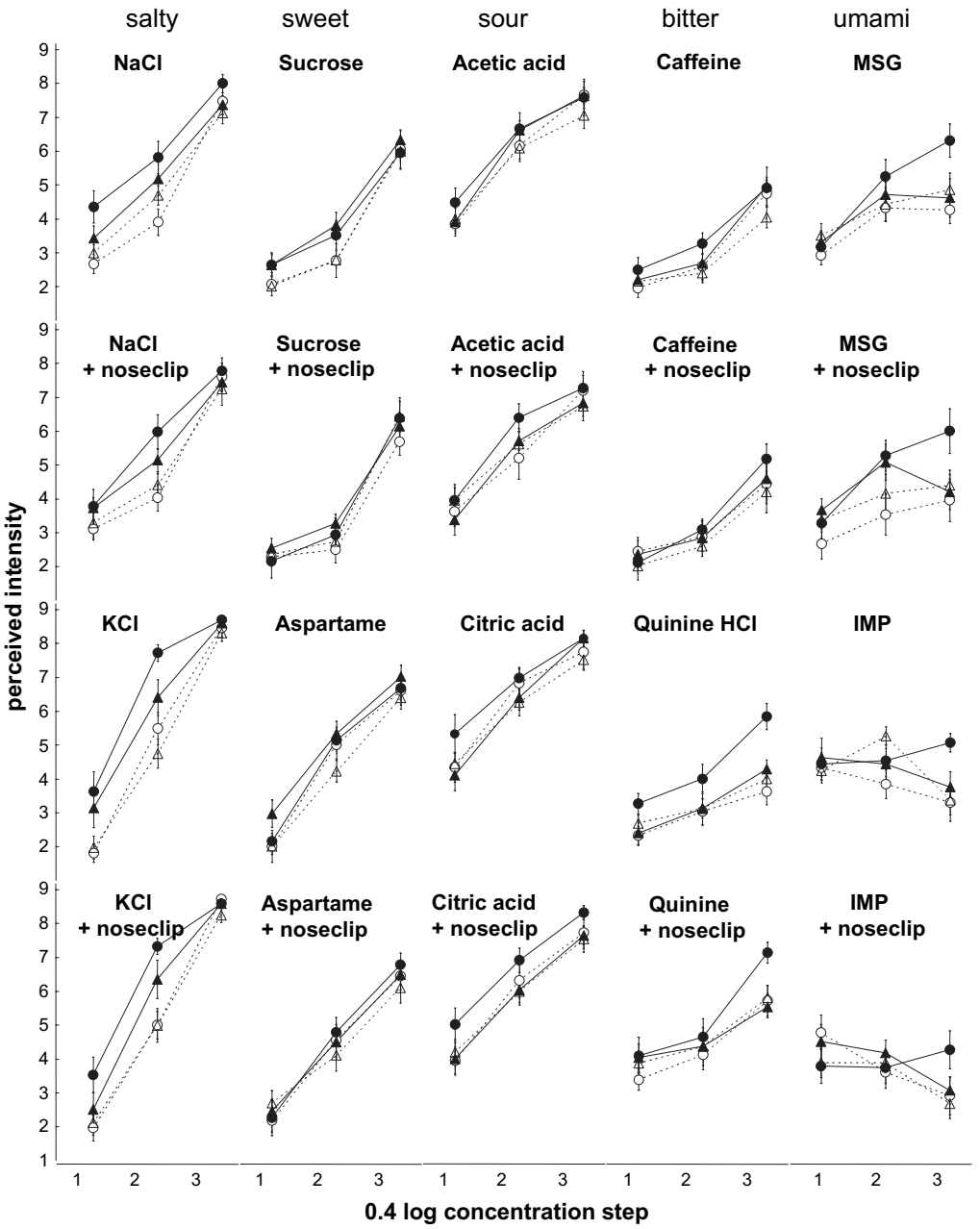

Figure 1 Intensity ratings (mean ± SE) for the ten tastants dissolved in water and assessed while wearing a noseclip (+noseclip) or not by elderly and young male and female subjects. Elderly subjects are represented by dotted lines and open symbols, young subjects by solid lines and filled symbols. Men are indicated by triangles and women by circles.

concentrations have in the dynamic range of perceived intensities (floor effects for NaCl, sucrose, caffeine and quinine HCl; ceiling effects for KCl and acetic acid), but for the umami taste qualities (MSG and especially IMP) more erratic and inexplicable relationships between concentration and intensity seem to prevail. Secondly, it should be noted that analysed over all tastants, no significant age and gender effects or age by gender interactions on intensity are found, although the elderly rated the saltiness of sodium chloride [$F(1,35) = 6.76$, $P < 0.02$] and potassium chloride [$F(1,35) = 18.57$, $P < 0.0001$] in water lower than did the young.

The intensities perceived by the group of young women are among the highest in almost all cases for salty, sour, bitter and for the highest concentrations of umami, but not for sweet, whereas the elderly usually perceived the tastants as less intense than the young (see Figure 1).

The noseclip effect on intensity

Since in the repeated-measures analysis of variance a main effect of wearing a noseclip was found [$F(1,35) = 10.21$, $P < 0.003$], the results of a further analysis of the differences in the intensities of the tastants perceived under the

conditions without and with a noseclip are given in Figure 2a, which shows the group means of the differences between the noseclip conditions, averaged per person over the three concentrations. As can be seen from this figure, in the majority of the cases, older subjects perceive the tastants as less different under the two conditions than do young subjects. In fact, for the elderly only the differences found for acetic acid [$T(18) = 3.81$; $P < 0.002$) and for MSG [$T(18) = 2.22$; $P < 0.04$) are significantly different from zero and the difference for IMP shows a trend [$T(18) = 2.03$; $P < 0.06$] in the same direction. Measured over all tastants, no significant deviation from zero is found for the elderly [difference = 0.11; $T(18) = 1.35$; $P < 0.20$] and a separate analysis for each of the three concentration levels over all tastants shows only a significant difference from zero [$T(18) = 2.37$; $P = 0.03$] for the middle concentration.

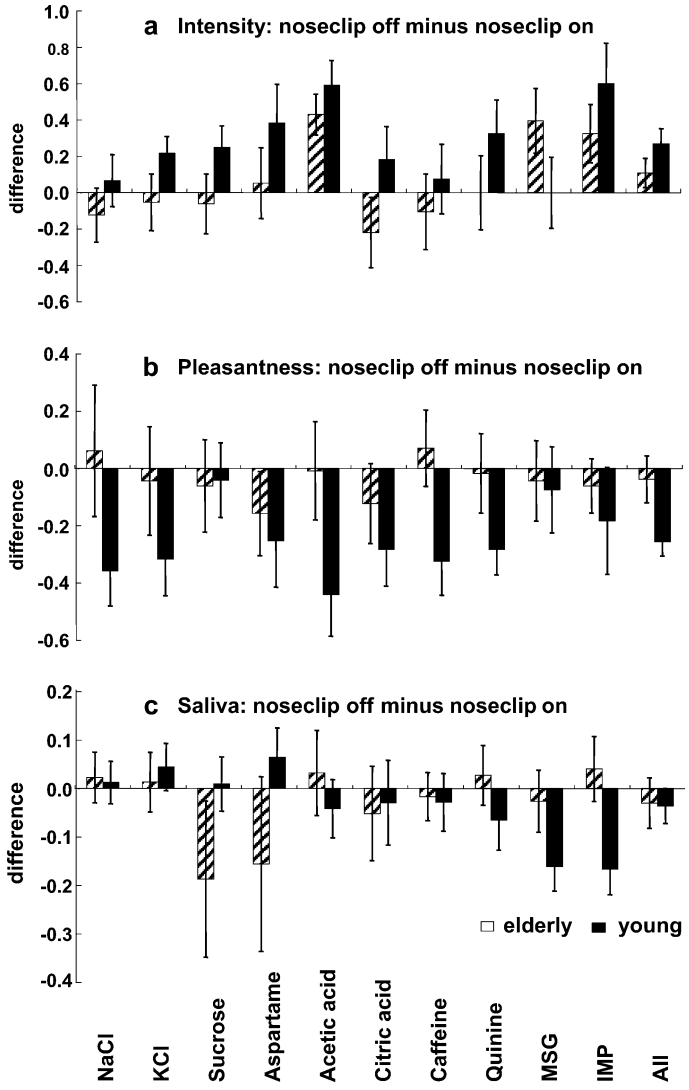

Figure 2 Differences (mean ± SE) between the unblocked and noseclip on (+noseclip) condition for elderly and young subjects assessing the ten tastants on intensity **(a)**, pleasantness **(b)** and amount of saliva **(c)**. Positive differences mean higher ratings of intensity/pleasantness or larger amounts of saliva were obtained in the noseclip off than in the noseclip on condition. Negative differences mean that the reverse was true. Elderly are represented by striped bars and young by filled black bars.

For the young, the mean difference over all tastants deviates significantly [difference = 2.69; $T(19) = 3.25$; $P < 0.005$] from zero and such significant differences are also found for the data over all compounds at each of the three concentration levels used [lowest concentration: difference = 0.23; $T(19) = 2.15$; $P < 0.05$; middle concentration: difference = 0.31; $T(19) = 2.98$; $P < 0.008$; highest concentration: difference = 0.27; $T(19) = 3.10$; $P < 0.006$]. Furthermore, in the case of the individual compounds, significant differences from zero are found for KCl [$T(19) = 2.33$; $P < 0.04$], sucrose [$T(19) = 2.12$; $P < 0.05$], acetic acid [$T(19) = 4.37$; $P < 0.0003$] and IMP [$T(19) = 2.71$; $P < 0.02$], while for aspartame [$T(19) = 1.81$; $P < 0.09$] and quinine [$T(19) = 1.76$; $P < 0.10$] trends in the same direction are found. It should be noted that for the young all these significant differences and trends are positive, indicating that the intensity of the tastants is reduced by wearing a noseclip. That this reduction is larger for the young than for the elderly means that, by putting a noseclip on, the difference in intensity perception between the two groups is reduced by about 70% (57.6% in men and 82.3% in women).

Pleasantness

In order to check whether the unpleasant feeling of wearing a noseclip might have negative effects on the appreciation for the stimuli, liking for the stimuli was measured under both the 'without noseclip' and the 'with noseclip' condition. In Figure 2b the differences in appreciation between the two noseclip conditions are shown for each of the tastants and for all tastants pooled. The elderly appreciate the individual tastants about equally well under both conditions and none of the differences found for them do deviate significantly from zero. Overall, the young do appreciate the tastants less with the noseclip off than with the noseclip on [difference = −0.26, $T(19) = -5.22$, $P < 0.0001$], and for NaCl [$T(19) = -2.92$, $P < 0.009$], KCl [$T(19) = -2.48$, $P < 0.03$], acetic acid [$T(19) = -3.06$, $P < 0.007$], citric acid [$T(19) = -2.21$, $P < 0.04$], caffeine [$T(19) = -2.75$, $P < 0.02$] and quinine HCl [$T(19) = -3.19$, $P < 0.005$] this is also the case. No differences in appreciation are found for both sweet and both umami tastants. Furthermore, it is clear that wearing a noseclip improves the appreciation in the young, and that it reduces the gap in appreciation between the elderly and the young by 69.6% (mean appreciation without noseclip: elderly = 4.19, young = 3.88; with noseclip: elderly = 4.23, young = 4.13). In fact, putting on a noseclip did not lead to lower appreciation of the tastants in any of the two age groups, indicating that possible irritation by it had no ill-effects on the results.

Saliva

The basic data on the saliva production in the two age groups are given in Figure 3. It shows that saliva production is in all cases higher in the young than in the elderly and in many

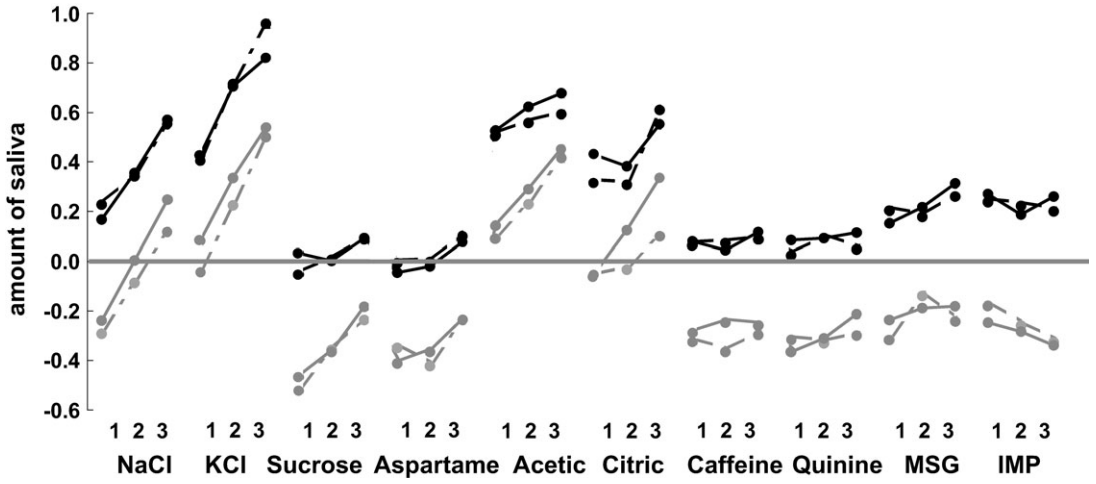

Figure 3 Amount of saliva for the three concentrations of each of the ten tastants for elderly and young subjects. The elderly are represented by grey lines, the young by black lines. Amounts produced with the nose clipped are shown by solid lines, while amounts produced with an unclipped nose are shown by broken lines. The amount of saliva is the difference between the weight of the cup before and after tasting and is expressed in grams.

cases the elderly obtain negative results, which might indicate that they suffer from a dry mouth and do not clear their mouth completely.

This is a systematic overall difference from the young, because the correlations between the amounts of saliva produced by the elderly and the young in response to the different concentrations of the different taste stimuli are $r = 0.950$ for the 'without noseclip' and $r = 0.953$ for the 'with noseclip' condition. This indicates that the salivation patterns to the different stimuli of the two groups are almost identical and that with regard to the quantity of saliva produced, the groups differ in the same way for all tastants and even for all levels of concentration of the tastants.

Furthermore, it is clear that the amounts of saliva produced are positively related to the concentrations of the tastants for the salty compounds NaCl [$F(2,34) = 48.31$, $P < 0.0001$] and KCl [$F(2,34) = 55.01$, $P < 0.000$], for the sweet compounds sucrose [$F(2,34) = 15.84$, $P < 0.0001$] and aspartame [$F(2,34) = 19.05$, $P < 0.0001$], for the sour compounds acetic acid [$F(2,34) = 9.34$, $P < 0.0007$] and citric acid [$F(2,34) = 24.28$, $P < 0.0001$], and to a certain extent for MSG [$F(2,34) = 7.92$, $P < 0.002$], but that this is not the case for the bitter compounds. For IMP a trend towards a negative relationship [$F(2,34) = 2.86$, $P < 0.08$] between the presented concentration and the saliva production is found. This latter finding is in line with the decline of the taste intensity with increasing concentration for this compound, as is described earlier in this section and shown in Figure 2. An interaction of concentration by age was found for sucrose [$F(2,340 = 3.35$, $P < 0.05$] where the saliva production of the elderly rose faster with the concentration of the tastant than that of the young, and for MSG [$F(2,340 = 3.30$, $P < 0.05$] where the reverse was true and the change in saliva production was very small for the older subjects.

The differences between the two noseclip conditions in saliva production by the elderly and the young, averaged over the three concentrations, are given per tastant in Figure 2c. For the elderly, none of these differences deviated significantly from zero. In some cases such as for the sweet tastants, the standard errors of the mean of the elderly were extremely large, indicating considerable individual variation in salivary production between members of the group for these tastants. For the young there were also no differences that significantly differed from zero, with the notable exception of the results for the two umami compounds. Both MSG [$T(19) = -3.22$, $P < 0.005$] and IMP [$T(19) = -3.18$, $P < 0.005$] invoked a larger saliva production in young subjects when they had their noses blocked.

It can be concluded that possible odorous effects of the classical taste stimuli (sweet, salty, sour and bitter) do not affect salivary function, but that umami differs from the other tastants in this respect.

Correlations

A correlation analysis was carried out to check whether the three types of data (intensity judgements, pleasantness judgements and saliva production) were related to each other. The correlations were calculated per age group over the individual responses of the subjects in each of the three tasks and calculated for all tastants pooled and for the separate tastants under each of the two noseclip conditions. For the elderly the only two significant correlations are found for the relationship between intensity and saliva production in response to NaCl under the 'with noseclip' ($r = 0.57$, $P < 0.01$) and 'without noseclip' ($r = 0.50$, $P < 0.05$) conditions, respectively.

For the young quite substantial negative correlations (all Ps < 0.01, unless mentioned otherwise) between intensity and liking were found under both noseclip conditions for the sour (without noseclip: $r = -0.76$ and -0.81; with noseclip: $r = -0.83$ and -0.74 for acetic and citric acid, respectively) and bitter stimuli (without noseclip: $r = -0.73$ and

−0.71; with noseclip: $r = -0.58$ and −0.62 for caffeine and quinine HCl, respectively) as well as for the salty stimuli under the noseclip 'on' condition (NaCl: $r = -0.69$; KCl: $r = -0.55$, $P < 0.02$) and to a lesser degree for the umami stimuli under the noseclip 'off' condition (MSG: $r = -0.50$, $P < 0.05$; IMP: $r = -0.66$, $P < 0.01$). For the sweet compounds no correlations between liking and intensity were found.

For the young the only significant correlation between intensity and saliva production was found for acetic acid under the 'without noseclip' condition ($r = 0.56$, $P < 0.01$). Negative correlations between liking and saliva production were found for both umami compounds when the nose of the young subjects was blocked (MSG: $r = -0.49$; IMP: $r = 0.46$, both Ps < 0.05).

When analysed over the individual average responses to the ten tastants, negative correlations between intensity and liking were found for the young under both noseclip conditions (without noseclip $r = -0.66$; with noseclip $r = -0.63$, both $P < 0.01$).

Preliminary conclusions

Like in earlier research (Mojet *et al*., 2003) with food products, wearing a noseclip reduced the difference in taste intensity perception between elderly and young for pure tastants dissolved in distilled water by ~70%. This strongly suggests that the differences in 'taste intensity' perception between elderly and young are predominantly due to differences in sensitivity to the smell rather than to the taste of the compounds. To accept the hypothesis that tastants themselves have a smell, direct proof is needed. It should also be shown that the effects are not due to changes in the olfactory quality of the solvent caused by cross-modal interaction with the tastant. Although it seemed unlikely that the water used had a sufficiently strong odour to explain the difference in reported 'taste' intensity between elderly and young, the precaution was taken to use three differently treated waters in the second experiment. In this experiment, the odours of tastant solutions were ortho-nasally tested, because it is easier to accomplish, avoids the risk of contamination by accidental taste stimulation in the mouth and because it is the most conservative measurement, since it has been shown (Zoeteman, 1978) that (mal)odorous compounds dissolved in water are retro-nasally detected at much lower concentrations than ortho-nasally. Thus, if the odours of the tastants in water are already discernible by ortho-nasal olfaction, their retronasal effects will be much stronger.

Experiment 2

Subjects

Forty-one subjects (13 men, age 28.2 ± 5.7 years; 28 women, age 30.8 ± 5.6 years) took part in the experiment. They were tested in four groups of about ten persons. All subjects were healthy and naïve with regard to the purpose of the experiment. They were recruited to judge water on the presence of possible odour contamination. At the end of the experiment they were paid for their participation.

Stimuli

For all ten tastants the highest concentration used in experiment 1 (see Table 1) was prepared in demineralized water, in double-distilled water and in commercially available Evian water. The samples were prepared on the evening before the experiment and kept at room temperature overnight. The next day 50 ml of each of the 30 solutions was transferred to a coded 150 ml plastic cup with an attached lid. Each of these stimuli was presented on a tray together with three similarly coded (random three-digit numbers), but otherwise identical cups containing only 50 ml of the same water as was used in the composition of the taste stimulus. The position of the target stimulus and the blanks was chosen at random with the restriction that over the three series all four positions of the target occurred about equally often. A separate randomized presentation order of the ten different stimuli was used in each of the three series (one series per type of water). The order of the series was balanced over the groups. Each of the series was first in one group, second in another group and third in the third group. The fourth group started with the double-distilled water, then received the Evian and ended with the demineralized water series.

Procedure

At the beginning of the session the subjects were seated independently around a set of large tables. Each subject sat in front of a tray with four coded cups (one stimulus and three blanks). In a four alternative forced-choice paradigm (4AFC) the subjects were asked to indicate which of the four cups smelled differently from the other three. They were told that often the task would be very difficult, but that they had to make a choice, even if they felt that it was only their best guess. At a sign from the experimenter, the subjects opened the cups, smelled the samples, noted their decision by crossing out a number on the response sheet, closed the cups again and passed the tray in the direction of their neighbour, who picked it up and waited for the next sign of the experimenter (interval between signs 60 s). This procedure was carried out ten times and followed by a pause of 5 min during which the stimulus sets and response sheets for the next series were distributed. The total time for completing the three series of the experiment was 45 min (including instruction).

Statistical analysis

Methods

For each taste stimulus the number of correct odour responses was calculated over the 41 subjects. This was expressed as a percentage and corrected for guessing by

the following calculation: percentage corrected = [(percentage observed – percentage expected by chance)/(100 – percentage expected by chance)] × 100. To find the number of subjects that did better than chance, the significance of the individual performances was first calculated using the binomial distribution (SAS Proc probbnml) at the individual level. With 30 observations, a chance probability of $P = 0.25$ and the possibility of obtaining from 0 to 30 correct responses an individual confidence level of 94.9% is met at 11 correct responses and a level of 97.8% at 12 correct responses. This means that 5% of the subjects who correctly detected 11 stimuli or more and 2.2% of those who detected 12 or more may have done so accidentally. Correcting the number of subjects that reached at least 11 correct responses by reducing it by 5% will therefore give a reasonable estimate of the number of subjects in the population that could detect the odours. Chi-square was used to verify whether the numbers of correct responses found for the three waters differed, and to verify whether the numbers of correct responses differed between the ten tastants used. The significance of the results obtained for the individual compounds was verified using the exact probabilities of the binomial distribution.

Effects with probabilities $0.05 > P < 0.10$ will be denoted as 'trends' or 'tendencies'.

Results

General

First the percentage correct responses to the stimuli were compared for men and women over all ten tastants and the three waters together. Men (37.2%) performed overall somewhat better [χ^2 (1) = 4.398, $P < 0.05$] than women (31.0%), and this was mainly due to the lesser performance of the women in the detection of quinine [men 33.3%, women 14.4%; χ^2 (1) = 4.849, $P < 0.05$]. Although in total the men outdid the women in seven out of the ten cases, none of the other differences were significant. Since the group of men was small ($n = 13$) and probably not representative of the male population, it was decided not to take too much notice of these possibly rather accidental gender differences and to pool the data of women and men in the further analyses. A correlation coefficient of $r = -0.0019$ between age and performance showed that age played no role in odour detection performance within the group.

The influence of the waters

Overall, no significant difference was found in the total numbers of correct responses (demineralized water 132, double-distilled water 147, Evian 126, $n = 410$) to the tastants when dissolved in the different waters [χ^2 (2) = 2.036, NS]. When the differences between the waters were tested for the individual tastants, there were also no significant differences, except in the case of IMP [demineralized water: 14, double-distilled water: 24, Evian: 6, $n = 41$; χ^2 (1) = 12.29, $P < 0.001$], whereas for MSG a similarly skewed but not significantly different distribution in the numbers of correct responses per water was found. When the results of the two compounds for each taste quality are combined, umami is also the only taste quality where the use of different waters leads to different results [demineralized water: 27, double-distilled water: 35, Evian: 11; χ^2 (2) = 12.29, $P < 0.001$]. This suggests that the use of double distilled water increases [χ^2 (1) = 13.67, $P < 0.001$] and the use of Evian water reduces [χ^2 (1) = 5.94, $P < 0.02$] the chance that the presence of umami odour is detected ortho-nasally.

Odour detection performance

The percentages odour detection after correction for chance guessing are given in Figure 4 for the whole group (all subjects), for the 19 subjects that had a correct score of 11 (binomial probability $P = 0.949$) or more out of 30 responses (11+ subjects), the 13 subjects that had a correct score of 12 (binomial probability $P = 0.978$) or more (12+ subjects) and the 3 subjects that detected the odour in 14 or more cases correctly (14+ group).

The total group performs significantly better than chance in detecting the odours of acetic acid which is detected in 93.5% (binomial $P < 0.001$), and IMP which is detected in 14.4% (binomial P < 0.01). For the odour of aspartame a detection trend is found at 8.9% (binomial $P < 0.10$).

The 11+ subjects, who, after correction for the 5% subjects that may have obtained a positive result accidentally, represent 44% of the total subject group, perceived the odours of the NaCl (binomial $P < 0.10$), sucrose (binomial $P < 0.01$), aspartame (binomial $P < 0.02$), acetic acid (binomial $P < 0.10$), citric acid (binomial $P < 0.10$), caffeine (binomial $P < 0.001$) and IMP (binomial $P < 0.01$) solutions in >10% of the cases after correction for chance guessing. The only tastant whose smell was never detected was KCl, whereas MSG and quinine HCl were detected only occasionally.

When the data of the 12+ subjects, who after correction for accidental results represent 29.3% of the total subject group, are considered, only the odours of three tastants (KCl, quinine and MSG) are *not* detected significantly more than could be expected by chance. NaCl and aspartame are both detected in 18.0% (binomial $P < 0.05$), citric acid, caffeine and IMP in 21.4% (binomial $P < 0.03$), sucrose in 31.6% (binomial $P < 0.003$) and acetic acid in 100 % (binomial $P < 0.001$). This means that after correction for chance guessing, ~30% of the subjects ortho-nasally perceive the odours of seven out of ten tastants dissolved in water at room temperature in at least 18% of the times they are presented to them.

The three most sensitive subjects of the 14+ group present a peculiar pattern of sensitivity. They are extremely sensitive to the odour of the solutions of sucrose, which they detect in 70%, and of NaCl and caffeine which they both detect in 40% of the cases, but they are somewhat less sensitive than the rest of the group to aspartame and IMP, whereas they show a significantly negative recognition of KCl, which, since it occurs

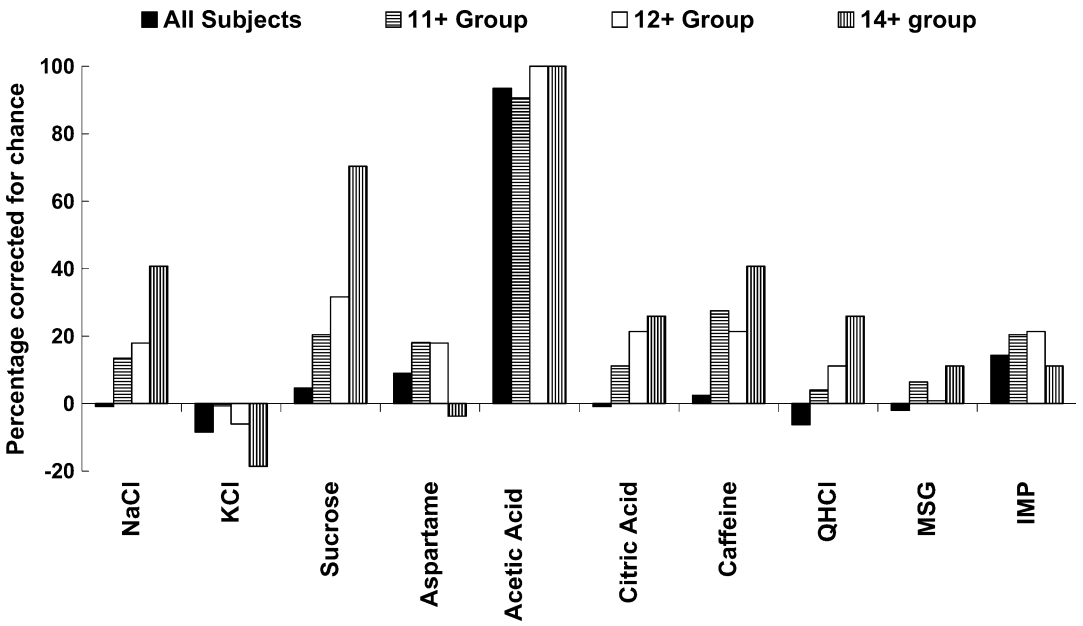

Figure 4 Percentage detection corrected for chance for the ten tastants for all subjects (n = 41, filled black bars) and for the subjects who had a correct score of 11 or more out of 30 responses (11+ subjects, n = 21, horizontally striped), of 12 or more (12+ subjects, n = 13, unfilled) and of 14 or more (14+ subjects, n = 3, vertically striped).

for all three waters (i.e. in three different stimulus sets) alike, cannot be the result of an artefact caused by accidentally smelling a control stimulus. This indicates that they do not detect KCl itself, but probably notice its presence through a reduction or a change in the odour of the aqueous solvents. This strengthens their conviction that this is not the stimulus containing the tastant and thus leads to a very low percentage of positive responses. Such a phenomenon is often observed when an alternative forced-choice method is employed (Sauvageot, 1984).

Of course it is not surprising that a selection of the subjects based on their superior response performance shows indeed a better performance, but an analysis of the differences in percentages corrected between the total group and the 12+ subjects shows that these differences are not evenly distributed over the ten tastants [χ^2 (9) = 51.45, $P < 0.01$] and this is also the case when the result of acetic acid, which left very little room for difference between the groups, is left out [χ^2 (8) = 45.14, $P < 0.01$]. This means that the differences between the total group and the 12+ group are, at least to a substantial part, due to the specific sensitivities of the 12+ group and not just to mere better luck in chance guessing.

Preliminary conclusions from experiment 2

The fact that seven of the ten tastants used can be detected by ortho-nasal olfaction and that this detection is independent of the water used for four of the five taste qualities strongly suggests that a number of tastants carry an odour and that the effects are not due to enhancement of the odour of the solvent. That the odour of umami was better perceived when dissolved in double-distilled water might perhaps be an indication of such an enhancement, but that would suppose that the double-distilled water had more of a different odour than the Evian water in which the umami was perceived less well. A simple check with a small group of experts did not show any detectable odour difference between these waters in triangle tests.

Discussion

Effects of olfactory deprivation

When tastants are dissolved in water, blocking olfactory cues by wearing a noseclip strongly reduces the differences between young and older subjects in taste intensity and/or taste liking, indicating that age-related differences in olfactory perception play an important role in 'pure' taste perception. Our finding that the difference in taste intensity ratings in this case was reduced by 70% instead of by the 100% as found in previous research (Mojet *et al*., 2003, 2004b) is due to the fact that some of the elderly in this experiment still had sufficient olfactory sensitivity to perform better without than with a noseclip. This is illustrated in Figure 5a–f, where the results of the experiments are compared for young and older subjects in the scatter plots of the correlation between the noseclip-off and the noseclip-on condition.

The story these figures tell is simple. For the young, the pattern of differences in taste intensity perception under the two noseclip conditions is indeed quite similar over the experiments, although the effects are substantially more pronounced when the tastants are dissolved in product. Most

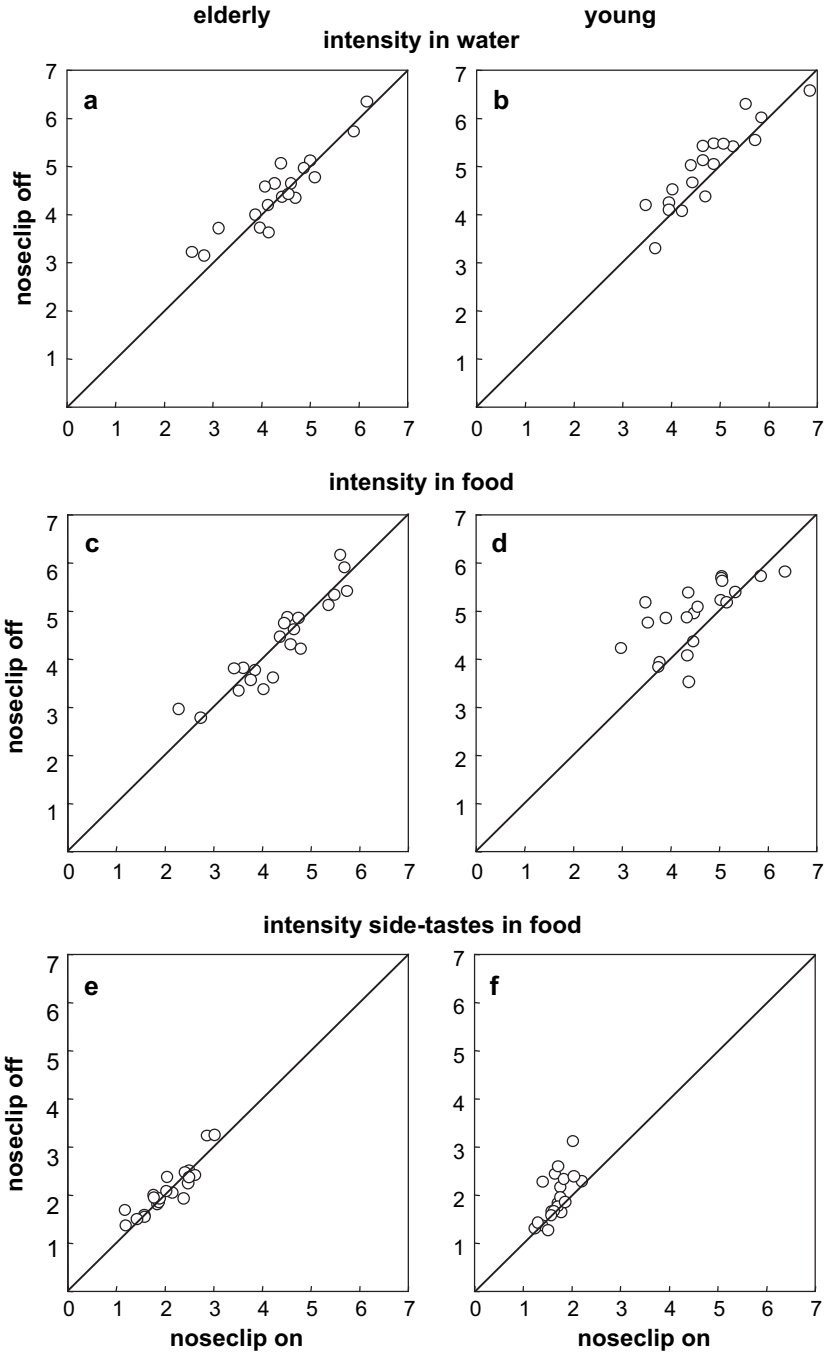

Figure 5 The effect of olfactory deprivation on the perceived taste intensities for elderly and young subjects. **(a, b)** The intensities in water of the present study. **(c, d)** The intensities of the experimentally varied tastants in food of a previous study (Mojet *et al.*, 2003). **(e, f)** The intensities of the side-tastes in food of a previous study (Mojet *et al.*, 2004b). Each point in the figure represents the average intensity rating over the ten tastants of a subject under the noseclip-on condition (*x*-axes) and noseclip-off condition (*y*-axes).

data points are found above the diagonal line, indicating that for most subjects the taste intensities are enhanced by olfactory cues. The elderly show the same effect, although to a lesser degree, when the tastants are judged in water, but when they are presented in product, the taste intensity judgements of the elderly do not seem to be influenced systematically by whether or not the subjects wear a noseclip. The difference between the noseclip effects in water and in product may of course be due to a lesser olfactory sensitivity of the elderly in the 'product group', but it may also be explained by assuming that the elderly find it easier to summate the odour and the taste intensity of the same tastant when they are perceived unmixed with other odours as in the water, but that they cannot do so when the odour of

the tastant is part of a complex mix that does not seem to be specifically related to the tastant in question. Schiffman (1979) also reported that aged subjects discriminated flavours less well than young subjects. Mojet *et al.* (2004b) found that their group of elderly systematically rated the intensities of the side-tastes (taste qualities that were only present at a low intensity in a product, e.g. sweetness in mayonnaise) higher than the young did, whereas they rated the intensity of the dominant (and experimentally varied) taste (e.g. sourness in mayonnaise) lower than the young. To explain this phenomenon, Mojet *et al.* (2004b) invoked a signal-to-noise ratio hypothesis and supposed that the elderly exhibited diminished quality discrimination ability, because they were less capable of separating sensory inputs. A similar phenomenon is known as the 'cocktail party syndrome' in audition and speech perception. In rooms where many people are talking, elderly people often have great difficulty listening specifically to the person who is talking directly to them.

In the same way, the odours of the tastants in the water condition stand out against a blank background, whereas in the product context they are swamped by other odours. It is unlikely that the group of subjects who judged the tastants in product ('product' group) was really much less sensitive than the subjects who judged the tastants in water ('water' group). This is illustrated by the fact that wearing a noseclip did have an effect on the liking of the elderly in the 'product' group (Mojet *et al.*, 2004a).

These findings and the fact that wearing a noseclip narrows the gap between the elderly and the young by about 70% for both intensity perception and liking are in good agreement with the well-documented fact that, compared to the other senses, the olfactory sense declines rather rapidly with age (Murphy, 1986; Doty, 1990; Doty and Laing, 2003).

The second question is whether the effects are due to odours given off by the tastants themselves, to impurities in the tastants or to interactions between the tastants (or their odours) and the odours of the solvent. Different waters were used in the second experiment to provide at least a partial answer to the last part of this question. Since, with the exception of the umami, for none of the tastants was a difference between the waters found, it seems unlikely that the odorous qualities of the waters were involved. Thus, it is clear that even with ortho-nasal olfaction the odours of the majority of the tastants themselves or of their impurities can be detected by almost a third of the young subjects in ~20% or more of the cases. Since retro-nasal stimulation by off-odours of water in the mouth (at ~35°C) has much stronger effects than ortho-nasal sniffing (Zoeteman, 1978), it can be safely assumed that a larger group of the young in the first experiment detected the odours of the tastants in a larger percentage of the presentations. In fact, it is possible to compare the reduction in intensity perception caused by the wearing of a noseclip for the ten tastants in the young group of experiment 1, with the amount of ortho-nasal detection of the tastants' odours in experiment 2 (only young subjects). A correlation of $r = 0.645$ [$T(8) = 2.71$, $P < 0.05$) between these two data sets is found, indicating that ~41% of the variance of the difference between the two noseclip conditions found for the different tastants in experiment 1 could be explained by variations in the ortho-nasal odour detectability of the tastants in experiment 2, notwithstanding the fact that two different groups of subjects were used. Thus, it might be that the retro-nasal perception of the odours of the tastants or their impurities is responsible for the differences between the two noseclip conditions in both age groups and that the difference between the two age groups in the extent of the noseclip effect is caused solely by the decline of the olfactory sensitivity of the elderly for these odours.

However, as indicated above, it should be realized that in young subjects the noseclip effects are stronger (see Figure 5a–d) when the tastants—and especially the dominant and experimentally varied tastants—are dissolved in product than when they are dissolved in water, whereas in the elderly this is not the case and the effects of noseclip are negligible. This again seems to confirm that the effects are due to the odour of the tastants or of their impurities for which the young are more sensitive than the elderly. Nevertheless it indicates at the same time that the the odours of the product themselves may interact with the tastants and provoke an enhancing effect. The fact that this only occurs for the young, who indeed perceive them as odorous, seems to suggest that this is in the first place an odour–odour interaction, the effect of which is then subsequently interpreted as a taste enhancement. Such an interpretation in terms of taste enhancement by odour is well in line with the literature on odour–taste enhancement described in the introduction (Frank and Byram, 1988; Stevenson *et al.*, 1999).

Saliva and umami

Although averaged over all tastants the young produce more saliva than the elderly, putting on a noseclip does not significantly change these amounts in either group for any of the salt, sweet, sour and bitter tastants (see Figure 3). This indicates that saliva production is not changed by the olfactory input introduced by the tastants. For the elderly this is also true for umami, but for the young blocking the nose significantly increases the flow of saliva at stimulation with both MSG and IMP. It can be concluded that the production of saliva, which contains itself MSG [~3.3 g/l according to Yamaguchi (1987), i.e. about a quarter of the highest 12.59 g/l stimulus and 1.5 times the lowest 1.99 g/l stimulus in this experiment], is decreased by the smell of the umami tastants. This is an interesting finding, because MSG is one of the two tastants that could not or only hardly be detected by ortho-nasal olfaction by the most sensitive (12+ and 14 + groups in Figure 4) of the young people in the second experiment and because MSG is the only tastant that is perceived

by the elderly, but not by the young, as more intense without than with a noseclip. This might suggest that the higher general saliva production of the young (independent of stimulation) leads to a higher general adaptation level for MSG, which makes it more difficult for them to detect its odour when stimulated with it, than for the elderly. Whether the rather strong presence of MSG in saliva is also related to the remarkable fact that only for umami were differences found in the ortho-nasal detection between the stimuli in the different solvents cannot be decided on the basis of the present results.

Conclusions

In conclusion, it can be said that, contrary to what is commonly assumed, all so-called 'pure tastants' used here—and in many experiments in the same or even less pure grades by others—are also olfactory stimuli and that most of the age-related 'taste' differences found are probably predominantly based on differences in olfactory sensitivity. This leads to the intriguing question as to what is smelled and how it is smelled. It is well known that acetic acid is volatile enough to be smelled at even rather low concentrations, but what about most of the other compounds? Is it possible that substances with no measurable vapour pressure still stimulate the nose? Or should the conclusion be that, even with the purest reagent or purissimum grade stimuli used in these experiments, the odours are due to impurities? The present experiment cannot provide an answer to these questions and although a first attempt to solve the problem by gas-chromatographic methods has not shown clear differences in volatile compounds between the headspace of the solutions and of the aqueous solvents, it is far too early to reach a conclusion. More refined experiments, combining gas-chromatography and retro-nasal psychophysical methods, should be carried out to clarify the precise causes of the olfactory stimulation, but in the meantime it should be realized that these findings have important consequences for the interpretation of the results of both animal and human taste research. Not only do they necessitate a reappraisal of many results on taste perception and taste memory, but above all they question the basis of many recent statements about the role of central mechanisms involved in odour–taste interaction and flavour perception.

Acknowledgements

The authors thank Nancy Holthuysen, Mariska Nijenhuis, Linda Huntjens and Kees van Kekem for their assistance in preparing and conducting the experiments and Dr J.H.A. Kroeze and two anonymous reviewers for their valuable comments on a previous version of this paper.

References

Bardow, A., Nyvad, B. and **Nauntofte, B.** (2001) *Relations between medication intake, complaints of dry mouth, saliva flow rate, saliva composition, and the rate of human tooth demineralization in situ*. Arch. Oral Biol., 46, 413–423.

Bradley, R.M. and **Beidler, L.M.** (2003) *Saliva: its role in taste function*. In Doty R.L. (ed.), Handbook of Olfaction and Gustation, 2nd edn. Marcel Dekker, New York, pp. 639–650.

Christensen, C.M. (1986) *Role of saliva in human taste perception*. In Meiselmann, H.L. and Rivlin, R.S. (eds), Clinical Measurements of Taste and Smell. Macmillan, New York, pp. 414–428.

Dawes, C. and **Watanabe, S.** (1987) *The effect of taste adaptation on salivary flow rate and salivary sugar clearance*. J. Dent. Res., 66, 740–744.

Doty, R.L. (1990) *Aging and age-related neurological disease: olfaction*. In Goller, R. and Graftnan (eds), Handbook of Neuropsychology. Elsevier, Amsterdam, pp. 211–226.

Doty, R.L. and **Laing, D.G.** (2003) Psychophysical measurement of human olfactory function, including odorant mixture assessment. In Doty, R.L. (ed.), Handbook of Olfaction and Gustation, 2nd edn. Marcel Dekker, New York, pp. 203–228.

Frank, R.A. and **Byram, J.** (1988) *Taste–smell interactions are tastant and odorant dependent*. Chem. Senses, 13, 445–455.

Froehlich, D.A., Pangborn, R.M. and **Whitake, J.R.** (1987) *The effect of stimulation on human parotid salivary flow rate and alpha-amylase secretion*. Physiol. Behav., 41, 209–217.

Guinard, J.X., Zoumas-Morse, C. and **Walchak, C.** (1998) *Relation between parotid saliva flow and composition and the perception of gustatory and trigeminal stimuli in foods*. Physiol. Behav., 63, 109–118.

Hornung, D.E. and **Enns, M.P.** (1984) *The independence and integration of olfaction and taste*. Chem. Senses, 9, 97–106.

Köster, E.P. (1968) *Relative intensity of odour mixtures at supra-threshold level*. Olfactologia, 1, 29–41.

Köster, E.P. (1969) *Intensity in mixtures of odorous substances*. In Pfaffmann, C. (ed.), Olfaction and Taste III. Rockefeller University Press, New York, pp.142–149.

Mojet, J., Heidema, J. and **Christ-Hazelhof, E.** (2003) *Taste perception with age: generic or specific losses in supra-threshold intensities of five taste qualities?* Chem. Senses, 28, 397–413.

Mojet, J., Christ-Hazelhof, E. and **Heidema, J.** (2004a) *Taste perception with age: pleasantness and its relationships with threshold sensitivity and supra-threshold intensity of five taste qualities*. Food Qual. Pref., in press.

Mojet, J., Heidema, J. and **Christ-Hazelhof, E.** (2004b) *Effect of concentration on taste-taste interactions in food for elderly and young subjects*. Chem. Senses, 29, 671–681.

Murphy, C. (1986) *Taste and smell in the elderly.* In Meiselmann, H.L. and Rivlin, R.S. (eds), Clinical Measurements of Taste and Smell. Macmillan, New York, pp. 343–371.

Murphy, C. and **Cain, W.S.** (1980) *Taste and olfaction: independence vs interaction*. Physiol. Behav., 24, 601–605.

Murphy, C., Cain, W.S. and **Bartoshuk, L.M.** (1977) *Mutual action of taste and olfaction*. Sens. Processes, 1, 204–211.

Murphy, C., Cain, W.S., Gilmore, M.M. and **Skinner, B.** (1991) *Sensory and semantic factors in recognition memory for odors and graphic stimuli: elderly versus young persons*. Am. J. Psychol, 104, 161–192.

Neyraud, E., Prinz, J. and **Dransfield, E.** (2003) *NaCl and sugar release, salivation and taste during mastication of salted chewing gum*. Physiol. Behav., 79, 731–737.

Pederson, A.M., Bardow, A., Beier Jensen, S. and **Nauntofte, B.** (2002) *Saliva and gastrointestinal functions of taste, mastication, swallowing and digestion*. Oral Dis., 8, 117–129.

SAS Institute Inc. (1989) SAS/STAT® User's Guide, Version 6, 4th edn. SAS, Cary, NC.

SAS Institute Inc. (1990) SAS® Procedures Guide, Version 6, 3rd edn. SAS, Cary, NC.

Sauvageot, F. (1984) Contribution à la caractérisation d'un groupe en évaluation sensorielle de denrées alimentaires. [Contribution to the characterization of a sensory evaluation panel for foods]. Thesis, University of Burgundy, Dijon, pp 416–431.

Schiffman, S. (1979) *Changes in taste and smell with age: psychophysical aspects*. In Ordy, J.M. and Brizzee, K. (eds), Sensory systems and communication in the elderly. Raven Press, New York, pp. 227–246.

Shaffer, G. and **Frank, R.A.** (1990) *An investigation of taste–smell interactions across four tastants and six odorants*. Chem. Senses, 15, 638.

Siegel, S. and **Castellan. J.N.** (1988) *Nonparametric statistics for the behavioural sciences*, 2nd edn. McGraw-Hill, Singapore.

Spielman, A.I. (1990) *Interaction of saliva and taste*. J. Dent. Res., 69, 838–843.

Stevenson, R.J., Prescott, J. and **Boakes, R.A.** (1999) *Confusing tastes and smells: how odours can influence the perception of sweet and sour tastes*. Chem. Senses, 24, 627–635.

Yamaguchi, S. (1987) *The umami taste*. In Boudreau, J.C. (ed.), Food Taste Chemistry. The American Chemical Society, Washington, DC, pp. 33–51.

Watanabe, S. and **Dawes, C.** (1988) *The effect of different foods and concentrations of citric acid on the flow rate of whole saliva in man*. Arch. Oral Biol., 33, 1–5.

Zoeteman, B.C.J. (1978) Sensory assessment and chemical composition of drinking water. Thesis, Utrecht University.

Accepted October 18, 2004