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A New Specific Ageusia: Some Humans Cannot Taste L-Glutamate

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Abstract

A new specific ageusia was found in human subjects for monosodium L-glutamate (MSG). Four tests were successively applied to discriminate non-tasters and hypotasters from tasters. (i) NaCl and MSG thresholds, and (ii) suprathreshold sensitivity were evaluated using the up-and-down procedure. Only 73% of 109 subjects common to both tests demonstrated a sensitivity for MSG significantly higher than their sensitivity to NaCl, and hence a specific sensitivity to L-glutamate. The remaining 27% who showed no significant difference in sensitivity to MSG and NaCl solutions were considered as putative hypotasters. (iii) Perception profiles (time–intensity) for MSG and NaCl were tested in 58 subjects and appeared significantly different in 47 tasters (81%). This technique helped in identifying among putative hypotasters of tests 1 and 2 a few tasters who perceived equal intensity for isoconcentration of NaCl and MSG but who could discriminate isomolar solutions on other cues. Thus, 19% of subjects, for whom no significant differences were found between MSG and NaCl time–intensity profiles, remained in the hypotaster group. (iv) A discrimination task including 24 triangular presentations per subject of NaCl and MSG 29 mM applied to the eight most severe hypotasters showed that two subjects at least (two of 58; 3.5%) could not discriminate between both stimuli. Moreover, these subjects probably perceived identical sensations for MSG and NaCl solutions. The six other hypotasters (10.3%) could discriminate both stimuli at the limit of significance. None of these eight subjects were able to identify the typical umami taste in 29 mM MSG.

Introduction

Monosodium L-glutamate (MSG) elicits a specific taste, which was named umami after Ikeda (Ikeda, 1912). MSG has two specific properties: a preference enhancer effect when added to foods, although MSG is not preferred in pure water (Yamaguchi and Takahashi, 1984; Roininen *et al.*, 1996), and a synergistic effect in mixtures with 5′-ribonucleotides such as inosine-, adenosine-, guanosine 5′-monophosphate, etc. (Yamaguchi, 1967).

Electrophysiological studies showed that several receptors should be involved in umami taste perception. The analysis of chorda tympani (CT) nerve responses to various ionotropic and metabotropic agonists of L-glutamate central nervous system (CNS) receptors (Faurion, 1991) suggested a similarity between taste and CNS glutamate receptors. The metabotropic L-glutamate receptors (mGluR4), and ionotropic L-glutamate receptors were further identified in circumvallate papillae taste cells of the rat by Chaudhari *et al.* (Chaudhari *et al.*, 1996). Moreover, the same authors showed that conditioned taste aversion to L-2-amino-4-phosphonobutyrate (L-AP4), an agonist of the CNS receptor mGluR4, generalized to L-glutamate in rats. Converging studies showed the involvement of at least two mGluRs and ionotropic receptors (Bigiani *et al.* 1997; Lin and Kinnamon, 1999; Sako and Yamamoto, 1999). More recently, Chaudhari *et al.* (Chaudhari *et al.*, 2000) identified the 'taste-mGluR4', a truncated form of the CNS mGluR4 receptor, as well as the original CNS form in circumvallate taste cells. Lin and Kinnamon (Lin and Kinnamon, 1999) showed by patch-clamp recordings that *N*-methyl-D-aspartate (NMDA) ionotropic receptors were present in the taste cells of fungiform papillae. Stapleton *et al.* (Stapleton *et al.*, 1999) on the one hand and Nakashima *et al.* (Nakashima *et al.*, 2001) on the other hand, studied by conditioned taste aversion the relative role of NMDA receptors in MSG taste transduction. Finally, it has also been suggested that L-glutamate might have a neurotransmitter function either at the synapse between taste cells and taste fibers (Lawton *et al.*, 2000; Caicedo *et al.*, 2000) or between taste bud cells (Caicedo *et al.*, 2000).

If several receptors to L-glutamate, ionotropic receptors, mGluRs receptors and maybe sweet sensitive receptors (Nakashima *et al.*, 2001), were actually involved in taste transduction, that is if the determinism of glutamate taste sensitivity were polygenic, we might find interindividual differences of quantitative sensitivity to MSG among subjects. Although studies already considered the evaluation of

threshold to MSG or glutamic acid compared with NaCl (Schiffman *et al.*, 1981, 1991; Yamaguchi, 1991; Mojet *et al.*, 2001) or the intensity functions recorded for these chemicals (Schiffman *et al.*, 1991; Yamaguchi, 1991), none considered individual sensitivities.

Population studies can reveal interindividual differences of sensitivity as in the case of phenylthiocarbamide (PTC) or 6-*n*-propyl-2-thiouracil (PROP). For these compounds, subjects can be classified as tasters or non-tasters, on a genetic heritable trait (Snyder, 1931; Lugg, 1966; Kalmus, 1971). Large interindividual differences in taste sensitivity were also shown for other organic compounds (Blakeslee and Salmon, 1935), which were demonstrated to be significantly higher than the intra-individual variance (Faurion *et al.*, 1980; Faurion, 1993; Froloff *et al.*, 1996). Understanding the glutamate taste receptor mechanism might benefit from a population study and the purpose of the present experiment was to look for a specific ageusia to L-glutamate. Psychophysical experiments were set up to study the distribution of the sensitivity to L-glutamate in human subjects. Non-tasters or hypotasters were distinguished from the taster population after a series of four successive experiments. The first two tests (detection threshold determination, suprathreshold evaluation of taste intensity) included a large number of subjects to screen for the possible incidence of hypotasters and the last two (time–intensity profiles and taste quality discrimination) verified this in a reduced part of the same sample.

Materials and methods

As MSG contains sodium, great care was taken to compare individual sensitivities to MSG relative to NaCl. Only subjects perceiving MSG at concentrations below their NaCl threshold concentration can be directly considered as tasters; however, we must also consider that a subject may perceive glutamate at concentrations equivalent to the perceived concentrations of NaCl. In this case, the evaluation of quantitative sensitivity cannot distinguish between a non-taster experiencing only the taste of Na^+ in MSG and a subject perceiving both, and therefore experiencing iso-intensity at isomolar concentrations. Those subjects, probably hypotasters to a variable degree, should not be considered as non-tasters. In order to sort out the tasters from the hypotaster group, we used the four tests described further below.

Subjects

Subjects came from the local university and the local community. A population of 171 subjects (98 women and 73 men, 25 ± 9 years old) participated in the threshold experiment. A population of 119 subjects (73 women and 46 men, 27 ± 10 years old) participated in the suprathreshold experiment. A common group of 109 subjects participated in both. Subjects performed one session per day, which lasted 90 min. Each subject fulfilled four sessions. They were asked not to eat, drink or smoke at least for 30 min before the session. Fifty-eight of 109 subjects also participated in the time–intensity test. Eight subjects with a very poor sensitivity to MSG compared with NaCl were included in the last discrimination task. Finally, 64 among the same group of subjects were tested for their sensitivity to PROP.

Stimuli

NaCl (Merck, France, MW 58) and MSG (Eurolysine, France, MW 187) concentrations ranged from 0.002 mM to 10 mM in a geometric progression of ratio 2 for threshold determination. For iso-intensity evaluation, the reference was 29 mM NaCl and MSG concentrations ranged from 0.12 to 60 mM in a geometric progression of ratio 2. Solutions were daily prepared in sterile conditions, using ultraviolet sterilized water (Aqua-Stoutz, Actini, France).

The time–intensity perception profile (finger-span) and the discrimination triangular tests were performed with 29 mM solutions of NaCl and MSG. PROP taster and non-taster subjects were discriminated with a 0.29 mM PROP solution (Sigma, France, MW 170.2), a concentration surely perceived by tasters (Drewnowski *et al.*, 1997).

Experimental procedures

Test 1: detection thresholds

Subjects tasted each pair, comparing the stimulus (NaCl or MSG) against water, without swallowing. They answered the question: 'which is not water?', rinsed their mouth with tap water, then rested. The concentration of the stimulus of the following pair was increased by one log to base 2 step up after a false response or decreased by one log to base 2 step down after a correct response. Subjects had to fulfill one paired test within no less than 30 s for NaCl threshold, and within no less than 50 s for MSG threshold to take account of the long-lasting perception of umami substances.

Thresholds were determined using the up-and-down procedure (U&D) of Dixon (Dixon and Massey, 1960; Faurion, 1993), associated with forced choice pair comparisons. It included five pairs after the first change of response from correct to false or vice-versa. The pattern of correct and false responses allowed determining a k coefficient in the Dixon's table (Dixon and Massey, 1960). The threshold concentration, T, was calculated according to the formula $T = C_f \times r^k$, with C_f being the final concentration presented and, r, the ratio between successive concentrations (geometric progression). The duration of one U&D test was ~7 min. During one session, each subject performed four U&D tests for NaCl and four for MSG threshold determinations and during the four sessions, 16 determinations of each.

Test 2: suprathreshold measurements

At suprathreshold level, the U&D technique was also used to evaluate the individual concentration of MSG eliciting an

isointense perception compared with the 29 mM NaCl reference. Subjects compared MSG concentrations versus 29 mM NaCl in forced choice pairs and answered the question 'which is stronger?'. They had to wait for a resting delay of 45 s after rinsing to take account of the long-lasting MSG perception so that each paired test lasted at least 1.15 min. The concentration of the stimulus (MSG) varied according to the U&D technique: increasing one log to base 2 step up if the stimulus had been evaluated as less strong as the reference (NaCl) and decreasing one log to base 2 step down if the stimulus had been evaluated stronger than the reference. The 29 mM NaCl isointense concentration of MSG, C_{iso} MSG, was calculated according to the formula C_{iso} MSG = $C_f \times r^k$. Each subject performed four U&D suprathreshold tests per session (16 in four sessions).

Both threshold and suprathreshold tests were computer assisted. Two laboratory-built automata based on Apple II computers and laboratory-made interfaces diluted stock solutions and delivered the right concentration to each of the eight subjects working simultaneously. Twelve U&D tests were run within the same session in the following order: NaCl thresholds, MSG thresholds, then suprathreshold isointensity evaluations, within 1.30 h. The concentrations were computer delivered according to the previous subject's response but the four U&D tests of the same nature were intermingled so that, for the subject, the concentrations looked randomized. The temperature difference between both stimuli within a pair was controlled and kept below 0.1°C.

Test 3: time–intensity profiles

A long-lasting lingering effect is usually observed for glutamate and, as a consequence, a long rinse is often necessary between pairs, contrasting with NaCl solutions. The time course of the perceived intensity for 29 mM solutions of NaCl and MSG was measured in 58 subjects of the initial group thanks to the finger-span technique (Berglund *et al.*, 1978), using a potentiometer in a circuit. The subject took a sip of the solution and, with closed eyes, continuously indicated the perceived intensity elicited by the stimulus. This intensity was evaluated as the distance between the subject's thumb, which was fixed on the potentiometer end and his/her forefinger tip attached to the moving cursor of the potentiometer. The voltage was digitalized, acquired and stored for further treatment. After a mouth rinse, the subject waited ~2 min between NaCl and MSG. The aim of this experiment was to observe a similarity or dissimilarity between the time–intensity profiles of both stimuli.

Test 4: discrimination test

At this step, putative hypotasters were subjects who seemed not to perceive L-glutamate anion in any of the first three tests. Triangular tests were used to check whether putative MSG hypotasters having passed the three preceding tests could discriminate NaCl and MSG at isomolarity (29 mM) on a qualitative basis. Eight subjects participated in a 24-triangle test presentation. Each triangle test was presented at a time interval of 1.30 min.

Statistical analysis

Threshold and suprathreshold evaluations

After four sessions, 16 NaCl detection thresholds, 16 MSG detection thresholds, and 16 MSG isointense concentrations were collected for each subject. The individual NaCl detection threshold, MSG detection threshold, and the MSG isointense concentration were calculated as means of 16 U&Ds, except in certain cases where the U&D data from first sessions were discarded if statistically different from the rest of the data (Student's t-test, $\alpha < 0.05$).

The distributions of NaCl and MSG thresholds and of MSG isointense concentrations were plotted on a logarithmic abscissae. Each distribution was compared with a normal theoretical distribution ($\alpha < 0.05$, χ^2). These distributions were studied with a multigaussian fitting analysis (Biopatch software, Biologic, Claix, France).

Statistical criteria for subject classification

Subjects exhibiting MSG sensitivity at isomolarity with NaCl were suspected not to be sensitive to the L-glutamate anion but only to the sodium cation in the MSG solution. The taster or hypotaster status was defined in the group of 109 subjects. In test 1, a subject was defined as a taster when his/her MSG threshold was lower than his/her NaCl threshold at the level of $P < 0.10$ (Student's t-test). This conservative criterion ($P = 0.10$) was selected in order to avoid the risk that tasters might be classified into the hypotaster category. On the contrary, borderline subjects were kept in the taster category. At suprathreshold level, the criterion chosen to classify a subject into the taster group was a MSG isointense concentration lower than the NaCl reference concentration (C_{iso} MSG + 2 SD < 29 mM NaCl).

The subject's classification was verified with the time–intensity test. The MSG time–intensity individual raw profile was normalized to the subject's maximal perceived intensity for NaCl (= unity). Profiles were then grouped into two categories according to the taster/hypotaster status of the subject after tests 1 and 2. A few hypotasters showing a difference between NaCl and MSG time–intensity profiles were reclassified into the taster group. Time intensity profiles for NaCl and MSG were averaged for each group of subjects, SEM were calculated. As the maximal intensity for NaCl was not localized at the same time depending on subjects, some variation remained in the NaCl group profile and the maximum of the averaged NaCl profile resulted lower than unity. Averaged profiles were finally smoothed with a sliding window on 5 s.

Student's t-test compared NaCl and MSG averaged time–intensity profiles at maximum intensity within each group of tasters and hypotasters (the degrees of freedom depending on the numbers of subjects). Similarly, tasters and hypotasters averaged time–intensity profiles were also compared

at maximum intensity. Student's *t*-test was also used for paired comparison of total averaged profiles (frequency of data acquisition: 1 Hz; time window superior to 200 s, d.f. > 200).

Finally, subjects were declared hypotasters if they exhibited a non-significant difference between NaCl and MSG sensitivities at both levels and if time–intensity profiles for NaCl and MSG were non-significantly different. Hypotasters were declared non-tasters if they met the β risk criterion at $P = 0.10$ for identity of perception for both stimuli in the triangular test (Table 1).

Results

Three examples of raw results are presented in Table 2 for tests 1, 2 and 4 and, in Figure 1, for test 3.

NaCl and MSG thresholds distributions

The group threshold for NaCl was 0.82 ± 1.05 mM (mean ± SD; $n = 171$) and the group threshold for MSG was 0.32 ± 0.35 mM (mean ± SD; $n = 171$). The distribution of individual values for MSG (Figure 2b), ranging from 0.006 to 2.89 mM, could not be fitted with a single normal distribution ($P < 10^{-8}$; χ^2; d.f. = 19). A multigaussian fitting

Table 1 Criteria and methods used for the classification of subjects (see Materials and methods for details)

	Test 1: detection threshold	Test 2: isointensity (reference = NaCl 29 mM)	Test 3: time–intensity MSG > NaCl	Test 4: discrimination test
Glutamate sensitive subjects	$T_{MSG} < T_{NaCl}$	$C_{MSG} < 29$	yes	yes: tasters
	$T_{MSG} < T_{NaCl}$	$C_{MSG} \approx 29$	yes	yes: tasters
	$T_{MSG} \approx T_{NaCl}$	$C_{MSG} < 29$	yes	yes: tasters
Glutamate less sensitive subjects	$T_{MSG} \approx T_{NaCl}$	$C_{MSG} \approx 29$	no	yes: hypotasters no: non-tasters

Table 2 Three examples for raw results in tests 1, 2 and 4

	Test 1: detection thresholds			Test 2: isointensity, C_{MSG} ± SD (mM)	Test 4: discrimination test, no. of correct responses
	T_{NaCl} ± SD (mM)	T_{MSG} ± SD (mM)	r (T_{MSG}/T_{NaCl})		
Taster: Mou	0.8 ± 0.7	0.4 ± 0.3	0.5	1.4 ± 1.1	24/24
Hypotaster: Urs	1.5 ± 1.1	1.4 ± 1.3	0.9	60 ± 0.0	14/24 $P < 0.05$[a]
Non-taster: Pal	0.2 ± 0.1	0.2 ± 0.3	1.1	55 ± 10.4	9/24 $P > 0.05$

[a]Triangle test for difference: critical number of correct answers [(Meilgaard *et al*., 1991) see p.338; CRC Press, Inc.].

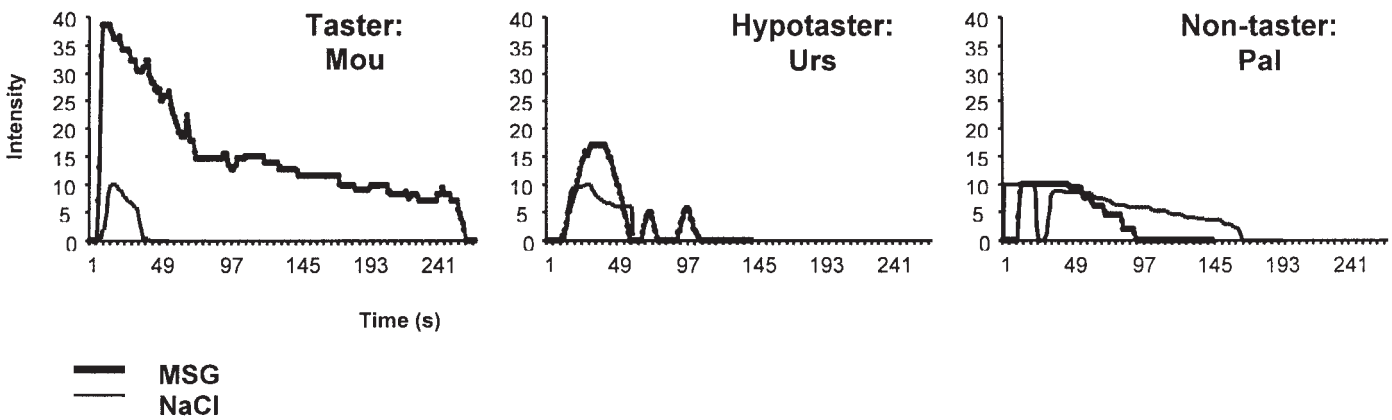

Figure 1 Three examples of individual time–intensity profiles. The time course of the perceived intensity was measured for NaCl (29 mM) and MSG (29 mM) with the finger-span technique. The units of perceived intensity are raw from digital acquisition.

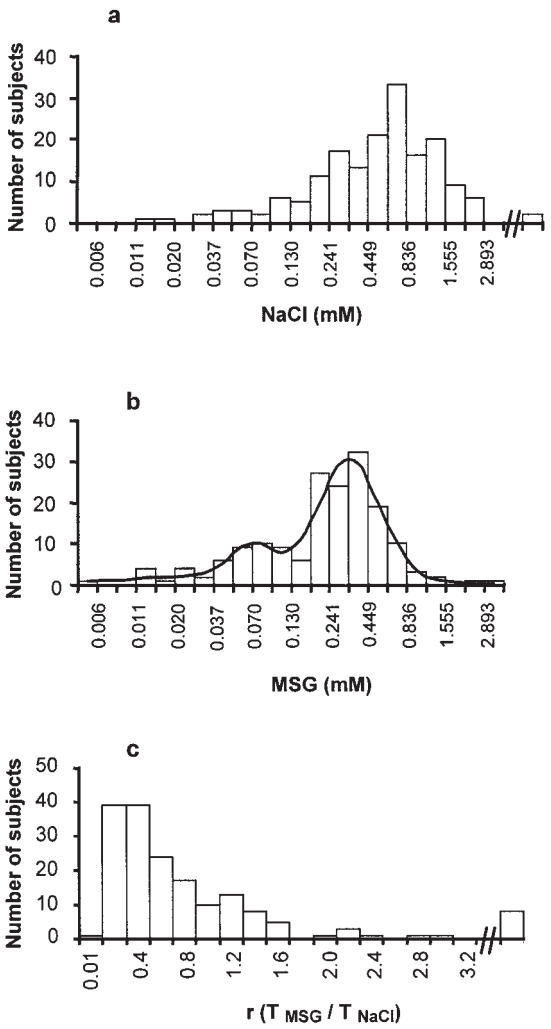

Figure 2 Distribution of individual NaCl and MSG thresholds and their ratios (n = 171). **(a, b)** Bin width of NaCl and MSG concentrations correspond to 0.135 log units, i.e. concentration $C_n = C_{n-1} \times 1.365$ mM. A multigaussian analysis disclosed at least two subpopulations for MSG thresholds (modes = 0.08 and 0.39 mM). **(c)** Ratios r of the MSG/NaCl threshold for each subject. Most subjects detected MSG at a lower concentration than NaCl ($r < 1$).

analysis (Biopatch software, Biologic) indicated at least two distinct normal distributions with respective means at m_1 = 0.08 mM (ranging from 0.03 mM to 0.18 mM: mean ± 2 SD) and m_2 = 0.39 mM (ranging from 0.14 mM to 1.07 mM: mean ± 2 SD). These subpopulations were statistically different (P = 0.03; t-test; d.f. = 155). Two subjects reached threshold values for MSG above 1.66 mM, i.e.: 3 SD above the higher mean threshold; and six subjects reached values below 0.02 mM, i.e.: 3 SD below the lower mean threshold m_1. Although the distribution of individual NaCl thresholds (Figure 2a) could not be fitted with a single normal function ($P < 10^{-4}$; χ^2; d.f. = 19), the multigaussian analysis could not reveal any clearly defined subpopulation for this stimulus.

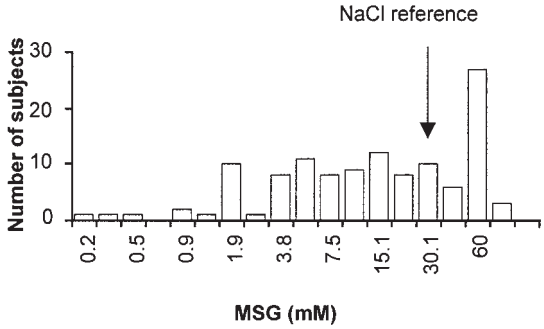

Figure 3 Distribution of C_{iso} MSG sensitivities, i.e. MSG concentrations perceived as isointense to 29 mM NaCl (n = 119). The arrow indicates isomolar concentrations. Subjects with a C_{iso} MSG not significantly lower than the 29 mM NaCl reference were classified as putative hypotasters.

Distribution of MSG sensitivity at suprathreshold level

Suprathreshold sensitivity for MSG was measured as the MSG concentration eliciting the same perceived intensity as 29 mM NaCl in 119 subjects. The group MSG isointense concentration was 22 ± 21 mM (mean ± SD). Although the distribution of isointense concentrations (Figure 3) was not fitted by a normal function ($P < 10^{-10}$; χ^2; d.f. = 15) subpopulations could not be established using multigaussian analysis.

Subject classification

Based on the statistical criteria established with the results of tests 1 and 2 (threshold and suprathreshold isointense evaluations), 80 of 109 subjects (73%) who perceived L-glutamate either at one or at both levels were classified as tasters. Of 80 subjects, 34 perceived L-glutamate at both levels (example 'Nic' in Figure 4), 28 showed they perceived L-glutamate specifically in the suprathreshold test only and 18 in the threshold test only (examples 'Mou' and 'Lab' respectively).

Table 3 exhibits the number of subjects that could be classified as putative hypotasters or non-tasters after each successive test. Figure 2c shows the ratio of individual threshold concentrations for MSG and NaCl ($r = T_{MSG}/T_{NaCl}$). Subjects with a ratio below unity perceived MSG at a lower concentration than NaCl and were easily discarded, after checking the significant difference of their average MSG and NaCl thresholds, from the group of hypotasters that was looked for in this study.

Twenty-nine of 109 subjects (27%) were classified as putative hypotasters considering their poor sensitivity to MSG at threshold level as well as at suprathreshold level. Among these 29 subjects, 19 showed a threshold for MSG not significantly different from their threshold to NaCl ($P \geq 0.10$; Student's t) and their C_{iso} MSG not significantly inferior to 29 mM, the concentration of the NaCl reference solution. Examples of data from such subjects are represented along the isomolarity line in Figure 4 (open symbols). The 10 other subjects among the 29 putative

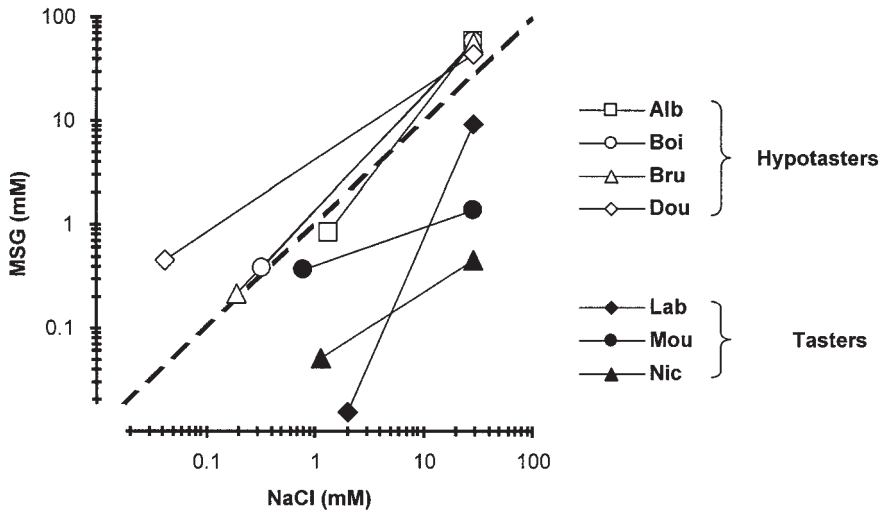

Figure 4 Relationship between NaCl and MSG threshold concentrations and C_{iso} MSG concentrations isointense to 29 mM NaCl (seven examples). Taster data ($T_{MSG} < T_{NaCl}$ and C_{iso} MSG < 29 mM NaCl) are localized below the dashed isomolarity line, hypotaster data on or above the line ($T_{MSG} \geq T_{NaCl}$ and C_{iso} MSG ≥ 29 mM NaCl).

Table 3 Summary of numbers of subjects and their respective classifications

	Tests 1 and 2: detection threshold and isointensity	Test 3: time–intensity MSG > NaCl	Test 4: discrimination test
Glutamate sensitive subjects	80 (73%)	47 (81%)	
Glutamate less sensitive subjects	29 (27%)	11 (19%)	hypotasters: 6 (10.3%) non-tasters: 2 (3.5%)
No. of subjects tested	109	58	8

hypotasters exhibited a significantly lower sensitivity to MSG than to NaCl (instead of a better sensitivity to MSG than to NaCl in tasters) either at threshold level ($n = 3$; $P < 0.04$; Student's t) or at suprathreshold level ($n = 7$; C_{iso} MSG –3 SD > 29 mM). These 10 subjects could not taste L-glutamate in the MSG solution; moreover, they perceived the sodium taste less well in the L-glutamate salt than in the chloride salt (example 'Dou' in Figure 4).

Independence of sensitivities to NaCl and MSG

The comparison of individual thresholds failed to reveal a correlation between the sensitivity to MSG and the sensitivity to NaCl ($r_s = 0.25$; $n = 171$; Spearman test). On the contrary, putative hypotasters alone clearly exhibited a positive correlation between MSG and NaCl thresholds ($r_s = 0.75$; $n = 19$; Spearman test).

Time–intensity profile

Two different time–intensity profiles for MSG were clearly observed within the group of 58 subjects among 109 who participated in the time–intensity test. Three examples of individual results are shown in Figure 1. The average tasters' time–intensity profile (Figure 5) was characterized by an eightfold difference between MSG and NaCl at maximum perceived intensity ($P < 0.001$; Student's t; d.f. = 92) and a long-lasting effect of 10 min or more for MSG without rinse. The overall paired (NaCl, MSG) profiles were significantly different ($P < 0.001$; paired Student's t; d.f. = 205). Conversely, the hypotaster category presented similar profiles for NaCl and MSG in intensity and duration of response (Figure 5). The time–intensity test confirmed hypotasters did not perceive MSG more intensely than the equimolar NaCl [Student's t = not significant (NS); d.f. = 20]. Their perception of MSG was significantly lower than the tasters' perception ($P = 0.003$; Student's t at 25 s; d.f. = 56).

After this test on 58 subjects, 47 subjects (81%) fell into the taster category and 11 subjects (19%) actually fell into the hypotaster category. Among the 47 tasters were eight subjects who had been previously classified among the putative hypotasters (Table 3). The time–intensity test showed they were able to discriminate both solutions, although they proved to be poorly sensitive on a quantitative basis in the first two tests.

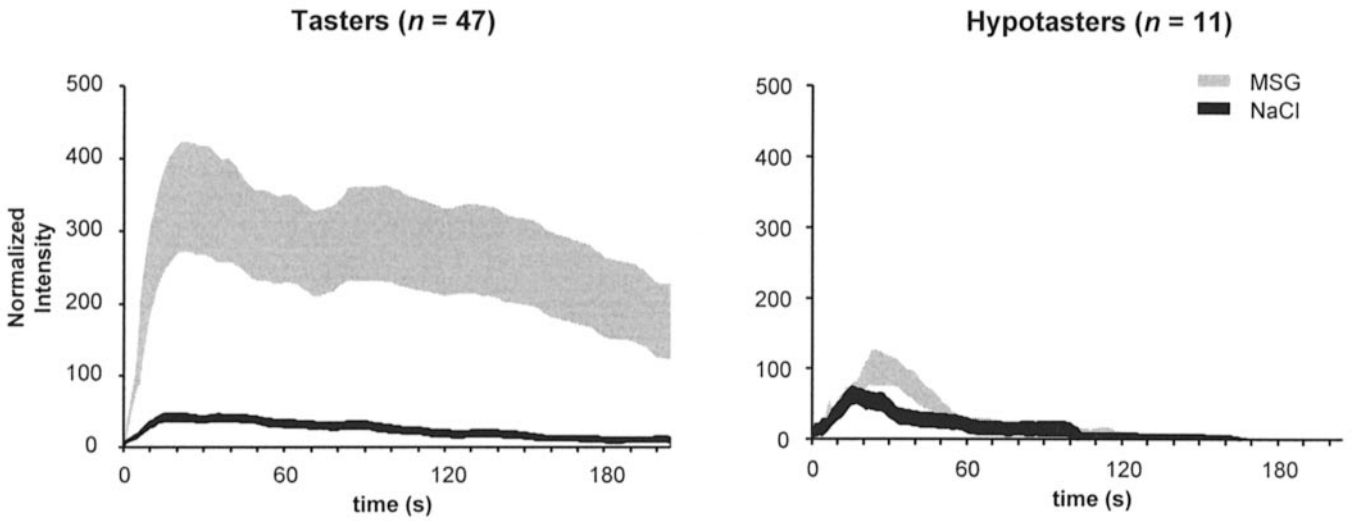

Figure 5 Mean range of time–intensity profiles of tasters and hypotasters. Profiles obtained for MSG were normalized for each individual relative to his/her NaCl maximal intensity (= unity). Individual profiles were then grouped and shaded areas indicate SEM on the group.

Discrimination test

A discrimination test was run to verify whether the preceding 11 hypotasters were also confusing MSG with NaCl at the same concentration as if the stimuli elicited the same quality. Eight among the 11 hypotasters of the preceding test performed the triangular discrimination test. Among them (Table 3), six subjects discriminated isomolar MSG and NaCl solution on a qualitative basis with 13–18 correct answers of 24 tests ($P \leq 0.05$, α error). These hypotasters, however, could not be considered as tasters in any of the first three tests. They presented a low score in the discrimination test compared with glutamate-sensitive subjects who clearly succeeded throughout this test. The two remaining subjects were assumed to be non-tasters as they could not discriminate MSG from NaCl (nine and 12 correct answers respectively of 24 tests; $P > 0.05$, α error). They met the statistical criterion of 0.10 for the β risk, which allows considering they perceived 'identical' tastes. The three subjects who missed this test remained undetermined either hypotasters or non-tasters and were not classified.

Subjects could be classified into the taster, hypotaster and non-taster categories after all four tests. Tasters represented 81% (47/58) of subjects. At least 10% (6/58) of subjects were considered as glutamate hypotasters and 3.5% (2/58) of subjects could be considered as glutamate non-tasters.

Comparison of MSG and PROP sensitivities.

Sixty-four subjects also tasted a 0.29 mM solution of PROP. The χ^2 calculated with a 2×2 contingency table on the number of PROP tasters/non-tasters and MSG tasters/hypotasters showed independence between MSG and PROP sensitivities ($\chi^2 = 0.12$; NS; d.f. = 1).

Discussion

The present study has shown a high interindividual difference of sensitivity for MSG as exhibited by distributions; the sensitivity to MSG is clearly continuous on the concentration axis. Comparing the individual sensitivity to NaCl and MSG allowed separating subjects into two groups: those who clearly discriminated MSG from NaCl because they perceived MSG at a significantly lower concentration than NaCl (tasters) and other subjects. The time–intensity and the triangular tests showed that among these other subjects, some could perceive MSG at iso-concentration with NaCl (hypotasters, 10%) and two subjects actually seemed to confuse MSG with NaCl (non-tasters, 3.5%). It is noteworthy that all these tests could be performed satisfactorily only after some learning (familiarization to MSG) and that no semantic description was found useful to pick out hypotasters in the population.

Working at constant intensity, with the U&D procedure, rather than at constant concentration, as with magnitude estimation, results in a very efficient method. The U&D procedure allows collecting several threshold or supra-threshold results per subject in a short time, which allows a statistical evaluation of the individual threshold, including the expression of the individual variance. The reproducibility of individual data can be checked across days and even within each session.

Distributions showed that the subjects of this study exhibited very different levels of sensitivity to MSG, thresholds ranging from 0.006 to 2.89 mM, and spanning a 500-fold range of concentration. Comparatively, the error on the individual subject's threshold was about twofold (with an average coefficient of variation of 0.9 ± 0.4, $n = 171$, for MSG and NaCl similarly), which is amazingly low

compared with interindividual differences. Hence, a large continuous variation of the sensitivity to L-glutamate was demonstrated in this population.

The absence of a correlation between MSG and NaCl individual thresholds shows the independence of both sensitivities in this group of subjects. Yamaguchi (Yamaguchi, 1991), Breslin and Tharp (Breslin and Tharp, 2001) and Mojet *et al.* (Mojet *et al.*, 2001) also stressed the independence of NaCl and MSG taste sensitivities. In the present study, hypotasters, on the contrary, showed a positive correlation between MSG and NaCl thresholds (r_s = 0.75). This showed that hypotasters probably perceived mostly the sodium cation in the glutamate salt.

It should be noted that detection threshold and suprathreshold results do not covary. Some subjects had similar threshold concentrations for MSG and NaCl but showed a MSG isointense concentration significantly lower than the 29 mM of the NaCl reference. Other subjects showed isointensity for isomolar solutions of NaCl and MSG but had MSG thresholds significantly lower than their NaCl threshold. Examples in Figure 4 showed that the sensitivity at threshold level could not predict the quantitative sensitivity of the same subject at suprathreshold level (and vice versa). This indicates a great difference in intensity functions among subjects as already shown in the literature (Faurion *et al.*, 1980). Schiffman *et al.* (Schiffman *et al.*, 1991) also showed different slopes of intensity functions for L-glutamic acid, in two groups of young and elderly subjects.

Both threshold and suprathreshold levels were necessary to determine the glutamate sensitivity status of a given subject. But qualitative tests also appeared necessary to discriminate MSG hypotasters and non-tasters from tasters, as exemplified in Table 3. Results from quantitative psychophysics lead to conclude that when subjects perceived isointensity at isomolarity, they may perceive only the sodium cation in the L-glutamate sodium salt. However, they might, on the contrary, be able actually to perceive L-glutamate at isomolarity with sodium. To overcome this uncertainty, we checked the ability of putative hypotasters to discriminate suprathreshold isomolar solutions of MSG and NaCl either by the time–intensity test or on a qualitative basis with triangular presentations. The time–intensity technique discarded at once among hypotasters, subjects who could discriminate the umami taste from the sodium taste. Though, these subjects were neither able to name it nor describe it even after the psychophysical training.

We noticed that subjects used the finger-span technique as a semiquantitative measurement: hypotasters used the full scale of the finger-span for small differences of perception as well as did tasters for large differences of perception. This technique acted as a sensitivity difference amplifier.

In the triangular discrimination test, eight hypotasters could statistically discriminate the glutamate anion from the sodium cation in the monosodium glutamate salt, on a qualitative basis. However, they were actually hypotasters regarding their quantitative data and definitely unable to comment on any difference of taste quality between both solutions. Furthermore, they could not give correct responses to all 24 triangles. Two subjects who could not discriminate MSG from NaCl might even experience an identical perception for NaCl and for MSG: they were therefore considered as 'non-tasters'.

The study of a salt such as MSG presents a specific difficulty relative to the presence of a cation should it be Na^+ or K^+ or Ca^+, etc. The sensitivity to L-glutamate is measurable up to the limit of the subject's sensitivity to this cation (here NaCl).

Distribution multimodality

The MSG threshold distribution revealed that hypotasters constitute a continuous group between the tasters and the non-tasters. The multigaussian analysis revealed two taster populations of average thresholds at 0.08 mM and 0.39 mM, respectively. This latter value is similar to findings by Yamaguchi (Yamaguchi, 1991), Yamaguchi and Ninomiya (Yamaguchi and Ninomiya, 2000) who could localize MSG threshold at 0.6 mM and also showed a slightly lower threshold concentration for MSG than for NaCl in their group of subjects. Comparatively, Schiffman *et al.* (Schiffman *et al.*, 1991) showed a group threshold for MSG at 0.9 mM for young subjects. Recently, Mojet *et al.* (Mojet *et al.*, 2001) found MSG thresholds from young subjects to elderly men and women ranging from 1.3 to 2.5 mM. At the other end of the distribution found in this study, a few subjects appeared extremely sensitive to glutamate. Similarly, in the literature, the distribution of PTC sensitivity also appears more than bimodal: Lugg (Lugg, 1966) showed that the PTC sensitivity of a population of 500 subjects could be divided into six modes. Moreover, a population of hypertasters was clearly identified for PTC (Lugg, 1962), and for PROP (Bartoshuk *et al.*, 1996).

PROP [or PTC, the ageusia to PTC (Kalmus, 1971) and to PROP is one and the same] and MSG, are presently the only two representatives of taste blindness in humans. Both show a multimodal distribution of human taste sensitivities indicating a multireceptor mediation. However, the independence between PROP and L-glutamate sensitivities, found in the present study, further indicates these ageusiae to be independent and suggests the contribution of different receptors or transduction mechanisms to recognize these stimuli.

Besides all statistical criteria on which categories can be built, it remains unclear whether the concept of 'non-taster' can be used for two reasons. The Na^+ cation perception may hide a low, although existing, *umami* stimulation due to the L-glutamate anion with no resulting perception. Many tests are necessary in putative non-tasters to make sure that no perception is possible under all conditions. Moreover, within

the literature relevant to PROP ageusia, the definition of a PROP 'non-taster' itself is ambiguous, as PROP non-tasters can perceive PROP by increasing the concentration of the solution.

Most interestingly, at threshold level or suprathreshold level, some subjects among hypotasters apparently could not perceive the Na^+ cation as well in the MSG solution as in the isomolar NaCl solution. This may be due to a difference in the degree of dissociation of both salts, MSG being less dissociated than NaCl. But another interpretation may be kept in mind. Although inefficient to produce an umami perception or any other measurable perception, L-glutamate may interfere with some transduction process and inhibit the sodium sensation. Together with the synergy effect between MSG and ribonucleotides (Yamaguchi, 1967; Sato *et al.*, 1970; Torii and Cagan, 1980), this inhibitory effect might be a new cue for the understanding of receptor events.

Mechanistic hypotheses: multiple receptors for umami taste?

Using psychophysical measurements, electrophysiological recordings and molecular modeling on various sweet and bitter compounds, Faurion *et al.* (Faurion *et al.*, 1980), Faurion and Vayssettes-Courchay (Faurion and Vayssettes-Courchay, 1990), Faurion (Faurion, 1993) and Froloff *et al.* (Froloff *et al.*, 1996, 1998) suggested a multireceptor hypothesis in which several receptor sites of low specificity cooperatively recognize organic molecules. Shimazaki *et al.* (Shimazaki *et al.*, 1981) and Cagan (Cagan, 1986) convergingly documented with biochemical assays the low affinity and low specificity of molecular chemoreceptors for taste. Other arguments converging on the multireceptor hypothesis can be found in recent literature. Abe *et al.* (Abe *et al.*, 1993) found 60 clones coding for putative receptor proteins in taste cells. Bernhardt *et al.* (Bernhardt *et al.*, 1996) showed that different stimuli (sucrose and saccharin) of the same quality could use different transduction pathways, hence different receptors, which was further confirmed by Uchida and Sato (Uchida and Sato, 1997) with D-phenylalanine and D-tryptophan, and reviewed by Herness and Gilbertson (Herness and Gilbertson, 1999).

For MSG particularly, a polygenic mechanism of L-glutamate chemoreception could be responsible for the multigaussian distribution observed. Arguments from the literature suggest the existence of several MSG receptor types in taste cells (G protein coupled receptors) or several transduction pathways. (i) Both, the 'taste-mGluR4' receptor and the CNS form of the mGluR4 [known to decrease intracellular cyclic adenosine monophosphate (cAMP)] are present in taste cells (Chaudhari *et al.*, 1996, 2000). As several other forms of L-glutamate receptors are expressed in the CNS, the same might be true for taste cells. (ii) An NMDA-like receptor was confirmed by patch-clamp studies (Lin and Kinnamon, 1999). (3) From an another point of view, an increase of the cAMP and of IP_3 (Ninomiya *et al.*, 2000) were both found involved after MSG stimulation. On the contrary, Bigiani *et al.* (Bigiani *et al.*, 1997), Lin and Kinnamon (Lin and Kinnamon, 1998) and Brand (Brand, 2000) suggested that 'taste-mGluR4' couples negatively to a cAMP cascade. As Varkevisser and Kinnamon (Varkevisser and Kinnamon, 2000) suggested, different transduction pathways may interact within a single taste cell either at the level of second messengers or at the target channels of the basal membrane. (iv) Furthermore, converging arguments suggest the implication of gurmarin sensitive receptors in addition to ionotropic and metabotropic CNS-like receptors for MSG taste sensitivity (Sako and Yamamoto, 1999; Ninomiya *et al.*, 2000; Nakashima *et al.*, 2001). These authors conclude to the involvement of sweet sensitive receptors. However, the relationship between MSG and sucrose chemoreception is not yet clearly understood. If MSG increases cAMP in taste cells as sucrose does (Striem *et al.*, 1989), this would be sufficient to explain some partial similarities between electrophysiological responses to L-glutamate and some sweet stimuli (Yamamoto *et al.*, 1991; Nakashima *et al.*, 2001).

The present study, showing more than one mode in the distribution of taster MSG thresholds, suggests that L-glutamate may interact with a series of receptors at the surface of taste cells. The coding of the L-glutamate taste signal could result from the cooperation of these various receptors and, therefore, depending on genetic peculiarities, human subjects might perceive or not the proper umami taste. Glutamate is probably a good example of a 'tastant using more than one mechanism' (Herness and Gilbertson, 1999). Finally, the existence of non-tasters together with the already accumulated knowledge on L-glutamate CNS receptors could be a very efficient entry into the study of taste receptors.

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References

Abe, K., Kusakabe, Y., Tanemura, K., Emori, Y. and **Arai S.** (1993) *Primary structure and cell type specific expression of a gustatory G protein coupled receptor related to olfactory receptors*. J. Biol. Chem., 268, 12033–12039.

Bartoshuk, L.M., Duffy, V.B., Reed, D. and **Williams, A.** (1996) *Supertasting, earaches and head injury: genetics and pathology alter our taste worlds*. Neurosci. Biobehav. Rev., 20, 79–87.

Berglund, B., Berglund, U. and **Lindvall, T.** (1978) *Separate and joint scaling of perceived odor intensity of n-butanol and hydrogen sulfide*. Percept. Psychophys., 23, 313–320.

Bernhardt, S.J., Naim, M., Zehavi, U. and **Lindemann, B.** (1996) *Changes in IP_3 and cytosolic Ca^{2+} in response to sugars and non-sugar sweeteners in transduction of sweet taste in the rat*. J. Physiol., 490, 325–336.

Bigiani, A., Delay, R.J., Chaudhari, N., Kinnamon, S.C. and **Roper, S.D.** (1997) *Responses to glutamate in rat taste cells.* J. Neurophysiol., 77, 3048–3059.

Blakeslee, A.F. and **Salmon, T.N.** (1935) *Genetics of sensory thresholds: individual taste reactions to different substances.* Proc. Natl Acad. Sci. USA, 21, 78–90.

Brand, J.G. (2000) *Receptor and transduction process for umami taste.* J. Nutr., 130, 942S–945S.

Breslin, P.A. and **Tharp, S.D.** (2001) *Reduction of bitterness and saltiness after a chlorhexidine rinse.* Chem. Senses, 26, 105–116.

Cagan, R.H. (1986) *Biochemical studies of taste sensation—XII. Specificity of binding of taste ligands to a sedimentable fraction from catfish taste tissue.* Comp. Biochem. Physiol., 85A, 355–358.

Caicedo, A., Jafri, M.S. and **Roper, S.D.** (2000) In situ *Ca^{2+} imaging reveals neurotransmitter receptors for glutamate in taste receptor cells.* J. Neurosci., 20, 7978–7985.

Chaudhari, N., Yang, H., Lamp, C., Delay, E., Cartford, C., Than, T. and **Roper, S.** (1996) *The taste of monosodium glutamate: membrane receptors in taste buds.* J. Neurosci., 16, 3817–3826.

Chaudhari, N., Landin, A.M. and **Roper, S.D.** (2000) *A metabotropic glutamate receptor variant functions as a taste receptor.* Nature Neurosci., 3, 113–119.

Dixon, W.J. and **Massey, F.** (1960) *Sensitivity experiments.* In: Introduction to statistical analysis. McGraw-Hill, New York, pp. 377–394.

Drewnowski, A., Henderson, S.A. and **Shore, A.B.** (1997) *Genetic sensitivity to 6-n-propylthiouracil (PROP) and hedonic responses to bitter and sweet tastes.* Chem. Senses, 22, 27–37.

Faurion, A. (1991) *Are umami taste receptor sites structurally related to glutamate CNS receptor sites?* Physiol. Behav., 49, 905–912.

Faurion, A. (1993) *The physiology of sweet taste and molecular receptors.* In Mathlouthi, M., Kanters, J.A., Birch, G.G. (eds), Sweet Taste Chemoreception. Elsevier Applied Science, London, pp. 291–316.

Faurion, A. and **Vayssettes-Courchay, C.** (1990) *Taste as a highly discriminative system: a hamster intrapapillar single unit study with 18 compounds.* Brain Res., 512, 317–332.

Faurion, A., Saito, S. and **Mac Leod, P.** (1980) *Sweet taste involves several distinct receptor mechanisms.* Chem. Senses, 5, 107–121.

Froloff, N., Faurion, A. and **Mac Leod, P.** (1996) *Multiple human taste receptor sites: a molecular modeling approach.* Chem. Senses, 21, 425–445.

Froloff, N., Lloret, E. and **Faurion, A.** (1998) *Cross-adaptation and molecular modeling study of receptor mechanisms common to four taste stimuli in humans.* Chem. Senses, 23, 197–206.

Herness, M.S. and **Gilbertson, T.A.** (1999) *Cellular mechanisms of taste transduction.* Annu. Rev. Physiol., 61, 873–900.

Ikeda, K. (1912) 8th International Congress of Applied Chemistry, Washington, DC and New York.

Kalmus, H. (1971) *Genetics of taste.* In Beidler, L.M. (ed.), Handbook of Sensory Physiology IV, Chemical Senses 2, Taste. Springer-Verlag, Berlin, pp. 165–179.

Lawton, D.M., Furness, D.N., Lindemann, B. and **Hackney, C.M.** (2000) *Localization of the glutamate-aspartate transporter, GLAST, in rat taste buds.* Eur. J. Neurosci., 12, 3163–3171.

Lin, W. and **Kinnamon, S.C.** (1998) *Responses to monosodium glutamate and guanosine 5′-monophosphate in rat fungiform taste cells.* In Murphy, C. (ed.), Olfaction and Taste XII. Annals of the New York Academy of Sciences, New York, 855, pp. 407–411.

Lin, W. and **Kinnamon, S.C.** (1999) *Physiological evidence for ionotropic and metabotropic glutamate receptors in rat taste cells.* J. Neurophysiol., 82, 2061–2069.

Lugg, J.W. (1962) *Some extremely high acuities of taste for phenylthiocarbamide.* Nature, 194, 980.

Lugg, J.W. (1966) *Taste thresholds for phenylthiocarbamide of some population groups. 3. The thresholds of some groups living in Japan.* Ann. Hum. Genet., 29, 217–230.

Mojet, J., Christ-Hazelhof, E. and **Heidema, J.** (2001) *Taste perception with age: generic or specific losses in threshold sensitiviy to the five basic tastes?* Chem. Senses, 26, 845–860.

Nakashima, K., Katsukawa, H., Sasamoto, K. and **Ninomiya, Y.** (2001) *Behavioral taste similarities and differences among monosodium L-glutamate and glutamate receptor agonists in C57BL mice.* J. Nutr. Sci. Vitaminol. (Tokyo), 47, 161–166.

Ninomiya, Y., Nakashima, K., Fukuda, A., Nishino, H., Sugimura, T., Hino, A., Danilova, V. and **Hellekant, G.** (2000) *Responses to umami substances in taste bud cells innervated by the chorda tympani and glossopharyngeal nerves.* J. Nutr., 130, 950S–953S.

Roininen, K., Lähteenmäki, L. and **Tuorila, H.** (1996) *Effect of umami taste on pleasantness of low-salt soups during repeated testing.* Physiol. Behav., 60, 953–958.

Sako, N. and **Yamamoto, T.** (1999) *Analyses of taste nerve responses with special reference to possible receptor mechanisms of umami taste in the rat.* Neurosci. Lett., 261, 109–112.

Sato, M., Yamashita, S. and **Ogawa, H.** (1970) *Potentiation of gustatory response to monosodium glutamate in rat chorda tympani fibers by addition of 5′-ribonucleotides.* Jpn. J. Physiol., 20, 444–464.

Schiffman, S.S., Sennewald, K. and **Gagnon, J.** (1981) *Comparison of taste qualities and thresholds of D- and L-amino acids.* Physiol. Behav., 27, 51–59.

Schiffman, S.S., Frey, A.E., Luboski, M.A., Foster, M.A. and **Erickson, R.P.** (1991) *Taste of glutamate salts in young and elderly subjects: role of inosine 5′monophosphate and ions.* Physiol. Behav., 49, 843–854.

Shimazaki, K., Sato, M. and **Takegami, T.** (1981) *Binding of [^{35}S]saccharin to a protein fraction of rat tongue epithelia.* Biochim. Biophys. Acta, 677, 331–338.

Snyder, R.H. (1931) *Inherited taste deficiency.* Science, 74, 151–152.

Stapleton, J.R., Roper, S.D. and **Delay, E.R.** (1999) *The taste of monosodium glutamate (MSG), L-aspartic acid, and* N*-methyl-D-aspartate (NMDA) in rats: are NMDA receptors involved in MSG taste?* Chem. Senses, 24, 449–458.

Striem, B.J., Pace, U., Zehavi, U., Naim, M. and **Lancet, D.** (1989) *Sweet tastants stimulate adenylate cyclase coupled to GTP-binding protein in rat tongue membranes.* Biochem. J., 260, 121–126.

Torii, K. and **Cagan, R.H.** (1980) *Biochemical studies of taste sensation. IX. Enhancement of L-[^{3}H]glutamate binding to bovine taste papillae by 5′-ribonucleotides.* Biochim. Biophys. Acta, 627, 313–323.

Uchida Y. and **Sato, T.** (1997) *Changes in outward K^{+} currents in response to two types of sweeteners in sweet taste transduction of gerbil taste cells*. Chem. Senses, 22, 163–169.

Varkevisser B. and **Kinnamon, S.C.** (2000) *Sweet taste transduction in hamster: role of protein kinases.* J. Neurophysiol., 83, 2526–2532.

Yamaguchi, S. (1967) *The synergistic taste effect of monosodium glutamate and disodium 5′-inosinate.* J. Food Sci., 32, 473–478.

Yamaguchi, S. (1991) *Basic properties of umami and effects on humans*. Physiol. Behav., 49, 833–842.

Yamaguchi, S. and **Ninomiya, K.** (2000) *The use and utility of glutamates as flavoring agents in food*. J. Nutr., 130, 921S–926S.

Yamaguchi, S. and **Takahashi, C.** (1984) *Hedonic functions of monosodium glutamate and four basic taste substances used at various concentration levels in single and complex systems.* Agric. Biol. Chem., 48, 1077–1081.

Yamamoto, T., Matsuo, R., Fujimoto, Y., Fukunaga, I., Miyasaka, A. and **Imoto, T.** (1991) *Electrophysiological and behavioral studies on the taste of umami substances in the rat.* Physiol. Behav., 49, 919–925.

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