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Responses of Single Taste Fibers and Whole Chorda Tympani and Glossopharyngeal Nerve in the Domestic Pig, *Sus scrofa*

Vicktoria Danilova, Thomas Roberts and Göran Hellekant

Department of Animal Health and Biomedical Sciences, University of Wisconsin, Madison, WI 53705, USA

Correspondence to be sent to: G. Hellekant, Department of Animal Health and Biomedical Sciences, University of Wisconsin, 1655 Linden Drive, Madison, WI 53705, USA

Abstract

Whole nerve, as well as single fiber, responses in the chorda tympani proper (CT) and glossopharyngeal (NG) nerves of 1- to 7-week-old pigs were recorded during taste stimulation. In the CT acids and in the NG bitter compounds gave the largest responses. Both nerves exhibited large responses to monosodium glutamate (MSG), MSG with guanosine 5′-monophosphate (GMP) and MSG with inositine 5′-monophosphate (IMP) as well as to glycine, xylitol, sucrose, fructose and glucose. Alitame, aspartame, betaine, neohesperedin dihydrochalcone (NHDHC), super-aspartame, saccharin and thaumatin elicited no or little response. Hierarchical cluster analysis of 49 CT fibers separated four major clusters. The M cluster, comprising 28.5% of all fibers, is characterized by strong responses to MSG, KCl, LiCl and NaCl. The responses to NaCl and LiCl were unaffected by amiloride. The H cluster (24.5%) includes units responding principally to acids. The Q cluster (18.5%) responds to quinine hydrochloride (QHCl), sucrose octaacetate (SOA) and salts with amiloride. The S cluster (28.5%) exhibits strong responses to xylitol, glycine and the carbohydrates as well as to MSG alone and to MSG with GMP or IMP. In 31 NG fibers, hierarchical cluster analysis revealed four clusters: the M cluster (10%), responding to MSG and MSG with GMP or IMP; the H cluster (13%), responding to acids; the Q cluster (29%), responding strongly to QHCl, SOA and tilmicosin^{R}; and the S cluster (48%), responding best to xylitol, carbohydrates and glycine but also to the umami compounds. Multidimensional scaling analysis across fiber responses to all stimuli showed the best separation between compounds with different taste qualities when information from both nerves was utilized.

Introduction

Anatomical studies show that, among the mammals studied, pigs have some of the highest number of taste buds (Chamorro *et al.*, 1993). Pigs have ~5000 fungiform taste buds (Chamorro *et al.*, 1993), compared with ~1600 in humans (Miller, 1986). The density of fungiform papillae is especially high along the rim of the pig's tongue, where they form a frill. Also, the posterior area of the pig's tongue has >10 000 taste buds in the vallate papillae, compared with 6000 in the human, and ~4800 in the foliate papillae, compared with 3000 in the human (Tuckerman, 1888). This means that pigs have 3–4 times as many taste buds as humans do.

Since studies in humans show a positive correlation between number of fungiform taste buds and ability to taste (Miller and Reedy, 1990), it is likely that pigs' sense of taste is not inferior to that of humans, but may in fact be superior.

Information from the fungiform taste buds is conveyed via the chorda tympani proper (CT) nerve. The terminology 'proper' is adapted from Langley (1898) to distinguish the chorda tympani innervating the sublingual and submaxillary glands from the one containing taste fibers. Langley used the term 'proper' for the chorda tympani with taste fibers.

Four studies have presented neural data from the pig CT (Kitchell, 1963; Hellekant, 1976a,b; Hellekant and Danilova, 1996). All four studies demonstrated a well developed ability to detect the standard taste stimuli: sucrose, NaCl, quinine hydrochloride (QHCl) and citric or acetic acid. On the other hand, monellin and thaumatin, two proteins that taste sweet to humans, as well as gymnemic acid and miraculin, two powerful sweet taste modifiers, had no gustatory effects in the pig (Hellekant, 1976a,b).

The glossopharyngeal nerve (NG) innervates mainly the vallate and foliate papillae. Little information has been published about the taste sensitivity of this nerve in the pig, but recordings from whole NG suggest that QHCl and sucrose were more effective stimuli than NaCl and acetic acid (Kitchell, 1963).

The scarcity of published taste studies and the growing importance of pigs as human-substitute models prompted this study. It presents results of electrophysiological recordings from both CT and NG during stimulation of the

tongue with ~30 compounds. Our selection of stimuli was guided by the choices in earlier studies and their use in pig feed or pig management.

Materials and methods

Animals and surgery

Domestic pigs (*Sus scrofa*) of both sexes, cross-bred Landrace × Large White × Duroc, ranging from 1 to 7 weeks in age and 1.5–15 kg in weight, were used. The CT responses were recorded from four animals ranging 1–3 weeks (not weaned) and eight pigs ranging 4–7 weeks (weaned). See Figure 3 for age distribution. The NG responses were recorded from 13 pigs ranging 4–6 weeks (weaned). The animals were housed at the University of Wisconsin farm. Each pig was anesthetized with i.m. injections of Telazol (6 mg/kg body wt) and Rompun (1 mg/kg body wt). The animal was then tracheotomized and put on halothane anesthesia (0.6–0.8%), which was maintained throughout the experiment. Body temperature, heart and respiratory rates were continuously monitored. Fluid was replenished with i.v. 5% dextrose in lactated Ringer's solution.

The right CT was dissected free from the point where it joins the lingual nerve to its exit from the tympanic bulla, where it was cut. It is relatively long and easy to dissect. The right NG was cut just peripheral to the petrosal ganglion and its peripheral part dissected free. The right angle of the mouth was cut to obtain access to the taste buds of the posterior tongue. The trenches of the foliate papillae were exposed by stretching the tongue.

Recording

In both nerves whole nerve and single taste fiber recordings were obtained. Nerve impulses were recorded with a PAR 113 amplifier, monitored over a loudspeaker and an oscilloscope, and fed into a recorder (Gould ES 1000) and into an IBM computer via a DAS-Keithley interface. For whole nerve recordings the nerve impulses were processed by a smoothed absolute value circuit integrator (Hellekant and Roberts, 1995) and changed to a DC potential whose amplitude was related to the nerve impulse frequency, here called the summated response. This signal and a code related to each tastant on the tongue were fed to the computer. Its program sampled the summated response before, during and after stimulation and displayed it on a monitor. It also controlled the stimulation times and the order of stimuli. The stimulus identity, its order, the maximum amplitude and integrated area of the response, the level of nerve activity before each stimulation and the time for each stimulation were continuously presented on the computer screen and printed out during the experiment.

For single fiber recordings the nerve was desheathed and teased into fine strands. Each strand was placed on a silver wire electrode held by a micromanipulator. An indifferent electrode was positioned in nearby tissue. The activity of single fibers was recorded with an impulse–amplitude analyzer. It had a window with adjustable upper and lower levels, and triggered a pulse when a nerve impulse exceeded the lower but not the upper level. These pulses were processed by the computer. Custom-made software controlled stimulus delivery and stored pulse interval data together with information on the presented stimulus (Hellekant and Roberts, 1995).

Stimulation

Solutions were delivered over the tongue by the computerized system previously described in detail (Hellekant and Roberts, 1995). The stimulation lasted for 5 s, with a 45 s rinse interval. The stimuli were dissolved in artificial saliva (Hellekant *et al.*, 1997a), which was also used as a rinse between stimulations. The stimuli and rinse were maintained and delivered at constant temperature (33°C) and flow (2 ml/s).

In both nerves we used essentially the same list of stimuli. Three concerns guided our choice of stimuli. Firstly, we included representatives of all four basic taste qualities. Secondly, we included compounds used in other species for comparison. Finally, we wanted to obtain taste nerve recordings of some of the additives used in pig food formula and diet. Since guanosine 5′-monophosphate (GMP) and inositine 5′-monophosphate (IMP) have been described as exerting synergistic effects on monosodium glutamate (MSG), we included mixtures of MSG with IMP or GMP in our stimulus array. Similar reasons underlie the mixing of thaumatin with saccharin or neohesperedin dihydrochalcone (NHDHC) (Higginbotham, 1994). The solutions and their concentrations are presented in Table 1.

Data analysis

The measure of the summated response was the integrated area of response during stimulation. It was calculated as the surface area under the trace and expressed in arbitrary units. The magnitudes of the summated responses were obtained by deducting the area of spontaneous nerve activity from that during stimulation.

The measure for the single fiber response was the number of impulses during the first 5 s of stimulation minus the number of spontaneous impulses recorded during 5 s of the prestimulus period. A fiber was considered to be responsive to a stimulus if the nerve impulse rate during the first 5 s of stimulation was more than two times the SD of the spontaneous activity of the fiber.

It may be suspected that gender may play a role. However, since no sex-related differences in taste nerve response have been reported in the literature we pooled our data. Further, our data gave no indication of any influence of gender.

The responses of the fibers to the four basic stimuli (NaCl, citric acid, QHCl and sucrose) were used to categorize each fiber by its best stimulus (Frank, 1973) and to calculate its breadth of tuning (H). H was calculated

Table 1 List of solutions used in two series of electrophysiological experiments in the domestic pig

CT series	NG series
NaCl, 0.1 M	NaCl, 0.1M
NaCl, 0.1 M + amiloride, 0.5 mM	
LiCl, 0.1 M	LiCl, 0.1M
LiCl, 0.1 M + amiloride, 0.5 mM	
KCl, 0.1 M	KCl, 0.1M
MSG, 70 mM	MSG, 70 mM
MSG, 70 mM + GMP, 3 mM	MSG, 70 mM + GMP, 3 mM
MSG, 70 mM + IMP, 3 mM	MSG, 70 mM + IMP, 3 mM
Citric acid, 20 mM	citric acid, 40 mM
Ascorbic acid, 40 mM	aspartic acid, 50 mM
Quinine HCl, 5 mM	quinine HCl, 5 mM
Caffeine, 0.15 M	caffeine, 0.1M
Denatonium benzoate, 0.3 mM	denatonium benzoate, 0.1 mM
Sucrose octaacetate (SOA), 1 mM	sucrose octaacetate (SOA), 1 mM
Cyclamate-Na, 22mM	Tilmicosin, 2%
Betaine monohydrate, 80 mM	betaine monohydrate, 80 mM
NHDHC, 0.5 mM	NHDHC, 0.5 mM
Sacc'n, 1.6 mM + NHDHC, 0.5 mM	sacc'n, 1.6 mM + NHDHC, 0.5 mM
Sacc'n, 1.6 mM	sacc'n, 1.6 mM
Sacc'n, 1.6 mM + thaum'n, 1.4 μM	sacc'n, 1.6 mM + thaum'n, 1.4 μM
Acesulfame-K, 3.5 mM	acesulfame-K, 3.5 mM
Stevioside, 0.87 mM	stevioside, 0.87 mM
D-Tryptophan, 0.03M	D-tryptophan, 0.03 M
Lactose, 0.6 M	lactose, 0.6 M
Maltose, 0.5 M	maltose, 0.5 M
Glucose, 0.5 M	glucose, 0.5 M
Galactose, 0.5 M	galactose, 0.5 M
Sucrose, 0.3 M	sucrose, 0.3 M
Fructose, 0.3 M	fructose, 0.3 M
Glycine, 0.4 M	glycine, 0.4 M
Xylitol, 0.6 M	xylitol, 0.6 M
Alitame, 0.3 mM*	
Aspartame, 5.3 mM*	
Super-aspartame, 0.11 mM*	

*Only in whole nerve recordings.

according to the formula $H = -Kp_i \log p_i$, where K is a scaling constant (1.6) and p_i is the proportional response to each of the four basic stimuli (Smith and Travers, 1979). Values of H range from 0 to 1. It is 0 when a fiber responds to only one of four basic stimuli and 1 when a fiber responds to all four.

Cluster and multidimensional scaling analyses were performed with the statistical package SYSTAT for Macintosh, version 5.2. Intercluster similarity was measured using the Pearson correlation and cluster analysis was performed according to the average linkage method. It should be noted that responses to all stimuli were used in these analyses.

The responses of the fibers belonging to the same cluster were first evaluated by two-way ANOVA on ranked data. This was followed by pairwise comparison of stimuli using Fisher's least significant differences. A probability of <0.05 was considered to be significant. To compare populations of similar fiber groups in the CT and NG we used a Pearson's Chi-square test.

Results

Chorda tympani nerve series

The whole CT nerve response

Figure 1 presents typical recordings of whole CT nerve activity. The stimuli have been arranged according to human qualities, i.e. salty, umami, sour, bitter and sweet. Both phasic and tonic responses are visible.

Figure 2 presents the averaged responses to all stimuli tested. The majority of the data were obtained from 12 animals, as shown by the number under each column. The most striking feature was large responses to acids, especially to citric and ascorbic acids. Interestingly, the acid responses always showed off-responses (Figure 1).

The second feature was moderate responses to NaCl and LiCl. These responses were not diminished by amiloride added to the salts. On the contrary, the responses to the mixtures were larger than those to the salts alone. For example, the average response to NaCl was 504.4 (± 43.7 SE) and the response to mixture of NaCl and amiloride was 754.3 ± 95.1.

The third feature was large responses to the umami compounds (MSG alone and with GMP or IMP). These responses cannot be attributed only to the sodium ions in the glutamate (70 mM), since the sodium in NaCl (100 mM) gave a smaller response.

The responses to the sweeteners varied from very large responses to glycine and xylitol to a hardly noticeable response to saccharin (Figure 1). If aspartame, super-aspartame, alitame, cyclamate and NHDHC gave any responses, they was barely noticeable.

Generally the bitter compounds elicited the smallest responses. Denatonium benzoate at 0.3 mM, a concentration that is very bitter to the human tongue, produced hardly any response in the two animals tested.

It may be surmised that the age of the animals affects their taste responses. Figure 3 presents the summated responses for different age groups. The upper diagram, depicting responses to the salts, MSG, QHCl and carbohydrates, suggests no consistent effect of age. In the lower diagram we combined data which may show an age-related effect (glycine, ascorbic and citric acids). The final conclusion will have to await a future study.

Single fiber responses in CT

The single fiber findings reinforce many of the observations in the summated recordings. This is evident in the overview of the responses of 49 fibers presented as a bubble chart in Figure 4. The stimuli were arranged along the x-axis in order of salt, umami, sour, bitter and sweet, while the fibers were arranged in order of best response to NaCl, citric acid, QHCl and sucrose. The area of the circles represents the impulse activity over the first 5 s of stimulation minus spontaneous activity before each stimulation. The legend included in Figure 4 relates circle area with nerve responses.

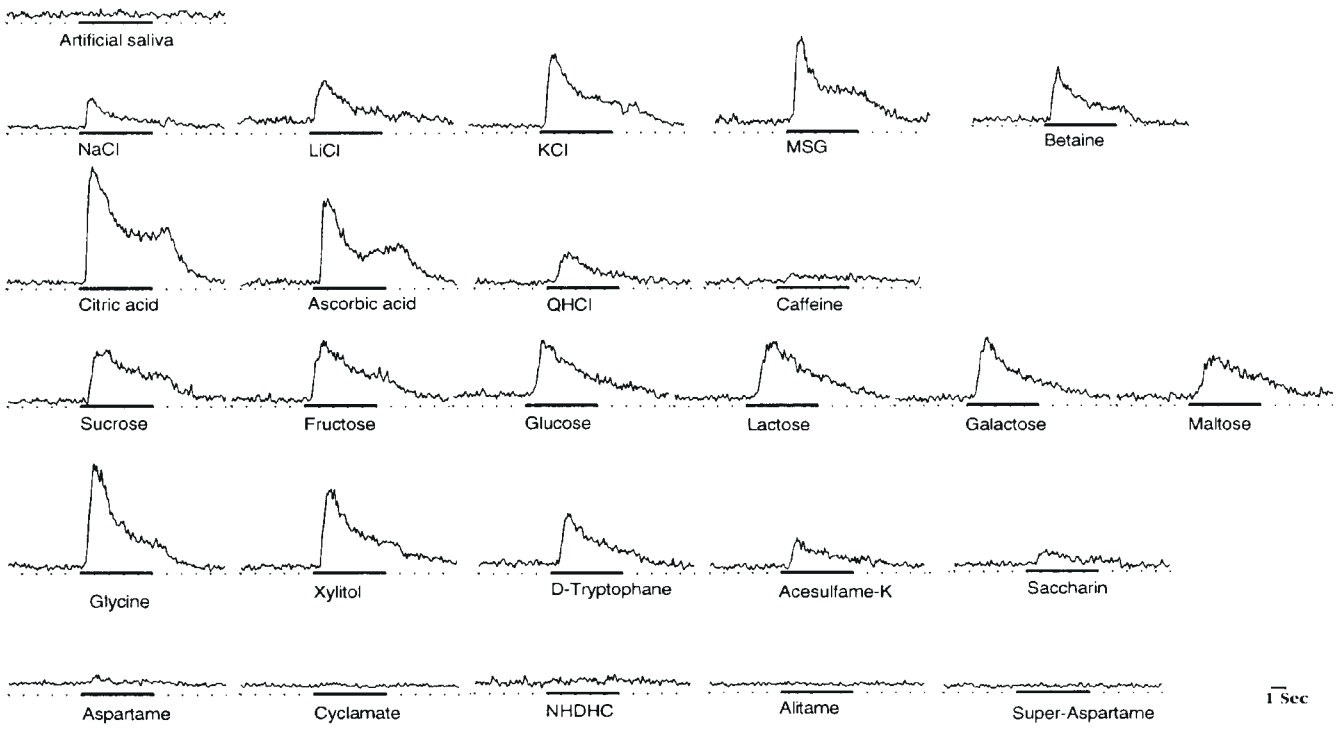

Figure 1 Summated CT nerve activity during taste stimulation of the tongue in a pig. The thick horizontal line at the bottom of each recording indicates the onset and end of stimulation.

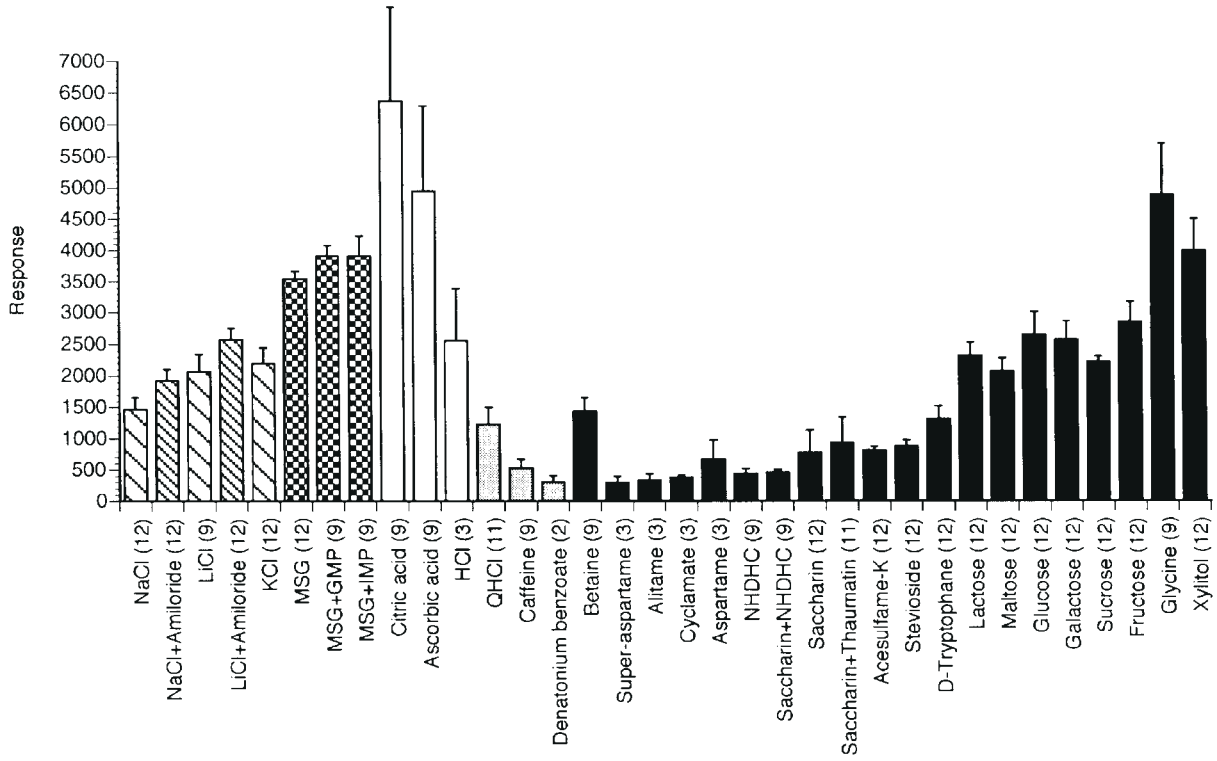

Figure 2 Averages of summated taste responses from the whole CT nerve to different stimuli. The columns represent the area of responses over the first 5 s of stimulation. Different taste qualities of stimuli (for humans) are coded by different texture in the columns. Hatched columns indicate salts; checkerboard, umami compounds; open columns, acids; grey, bitter compounds; and black columns, sweeteners. Numbers within brackets show number of animals tested with each compound. The error bars show the SE.

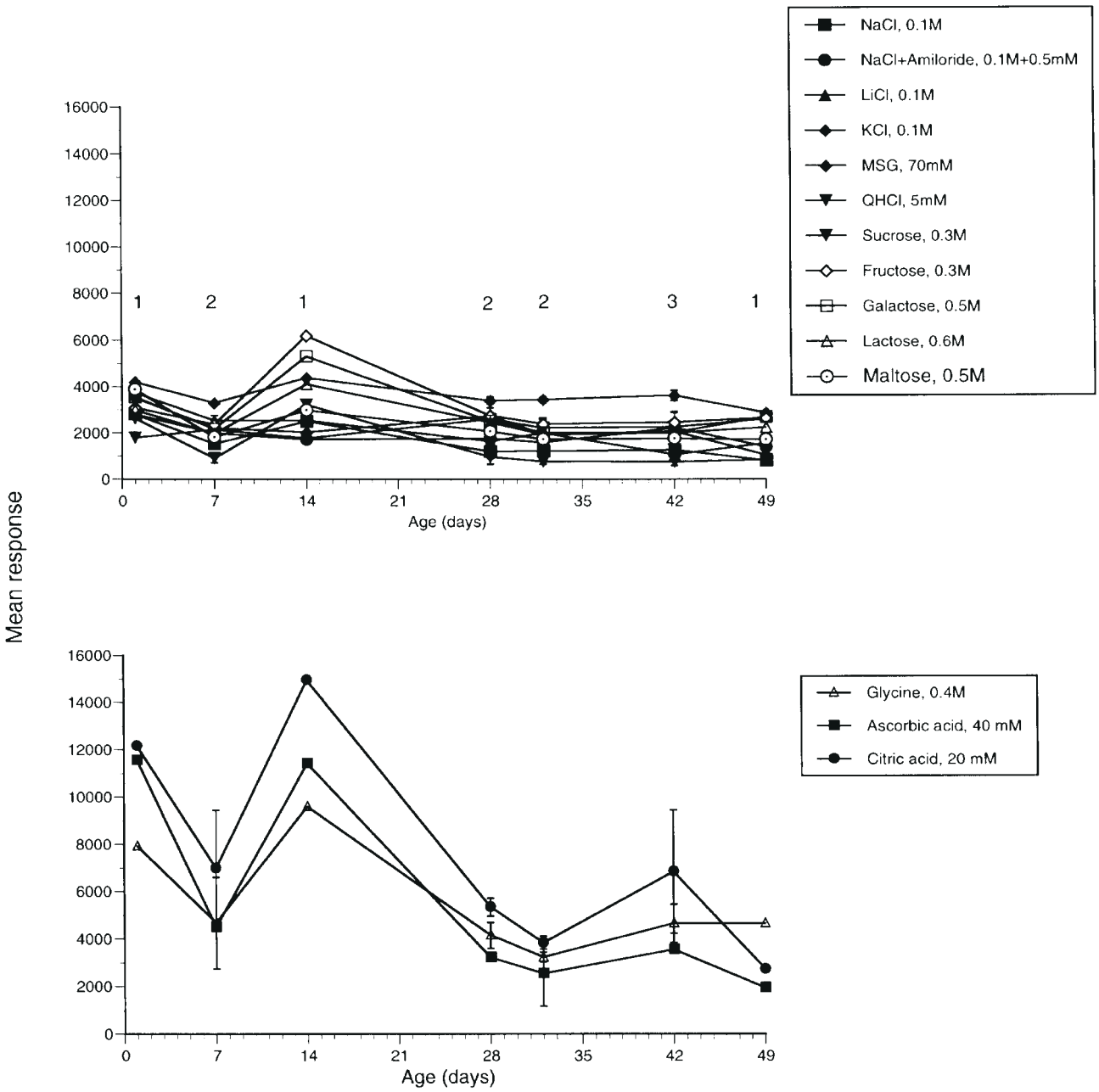

Figure 3 Average summated responses at different age. The numbers above the upper graph indicate the number of pigs in each age group.

Absence of a mark shows that data are missing. The average spontaneous activity of the 49 CT fibers was 12.0 ± 1.7 impulses/5 s.

It is obvious that the fibers responded to stimuli from more than one taste quality. This is also evident in Table 2, which shows the number of fibers in each best-stimulus group and their mean breadth of tuning (*H*). The group of acid-best fibers is most populous, while the group of salt-best fibers is smallest. The acid-best fibers were most narrowly tuned, while the QHCl-best fibers were most broadly tuned. The mean value of entropy for all the fibers was 0.62, which is higher than reported values for CT fibers of other mammals. This indicates that the specificity of taste fibers in the pig CT is lower than for other species and that the distribution of CT fibers of the pig conforms less with human taste qualities as they are represented by NaCl, sucrose, citric acid and QHCl.

Hierarchical cluster analysis

The responses of all fibers were subjected to hierarchical cluster analysis. We included the responses to all compounds

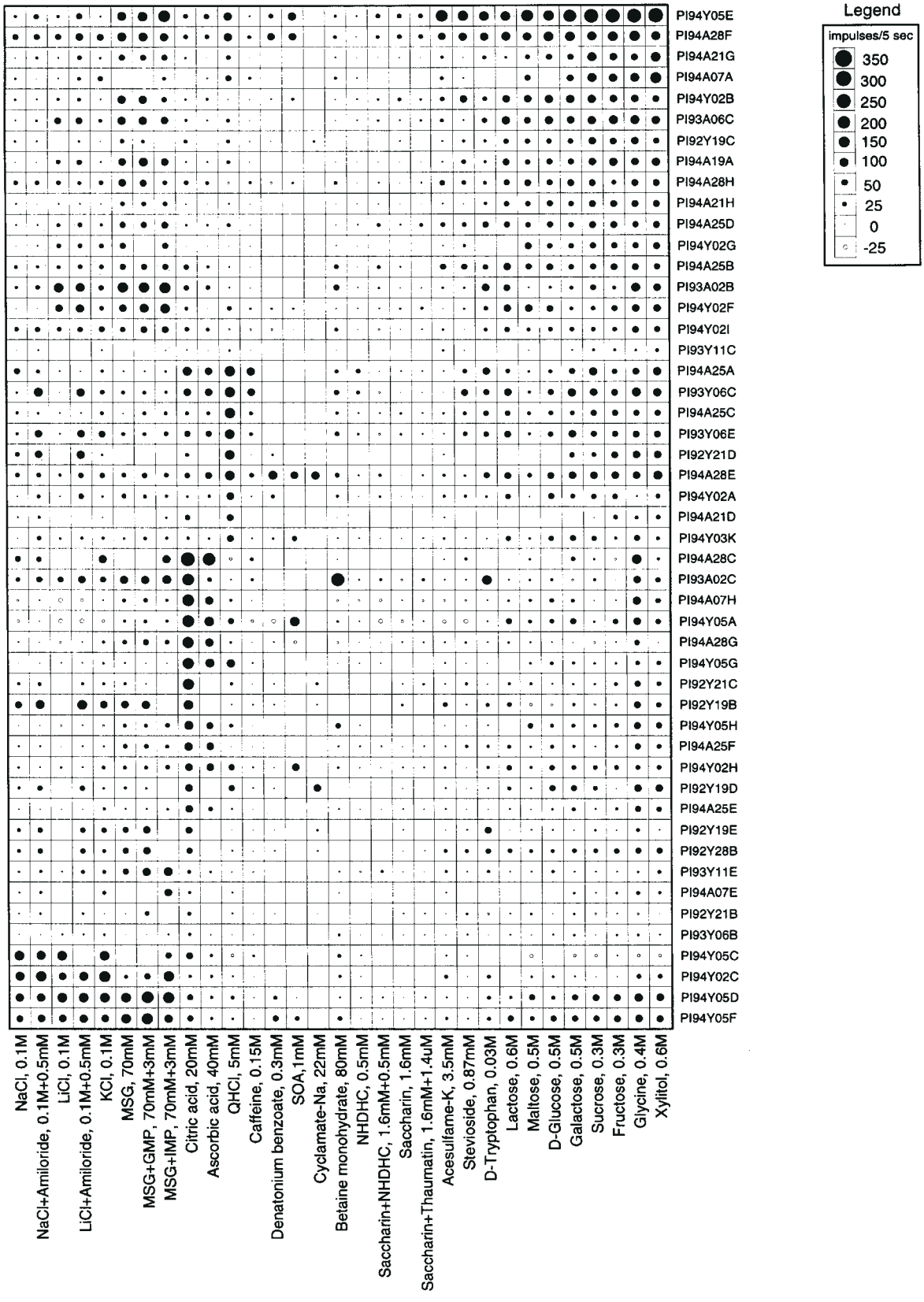

Figure 4 An overview of the response profiles of single CT fibers of the pig. The area of the circles represents impulse frequency over the first 5 s of stimulation. Open circles represent inhibition. Absence of a mark shows that data are missing. The stimuli were arranged along the *x*-axis in order of salt, umami, sour, bitter and sweet. The fibers were arranged along the *y*-axis in groups: NaCl-, citric acid-, QHCl- and sucrose-best fibers.

Table 2 Breadth of tuning and percentage of CT and NG fibers

	Number of CT fibers	Percentage	Mean (± SE) H for CT fibers	Number of NG fibers	Percentage	Mean (± SE) H for NG fibers
NaCl-best fibers	4	8.3	0.68 ± 0.15	0	0	–
Citric acid-best fibers	19	39.5	0.51 ± 0.05	3	9.7	0.7 ± 0.02
QHCl-best fibers	9	18.8	0.78 ± 0.04	8	25.8	0.47 ± 0.07
Sucrose-best fibers	16	33.4	0.64 ± 0.06	20	64.5	0.35 ± 0.04
All fibers	48		0.62 ± 0.03	31		0.41 ± 0.04

except SOA and cyclamate. The reason for this is that these two stimuli were used only in a few fibers.

The dendrogram in Figure 5 presents the result. Listed on the left is each fiber's number and response category based on its response to the four standard solutions. The cluster analysis separated four clusters: M, H, Q and S.

M cluster. As shown in Figure 5, the M cluster consisted of four NaCl-best, seven citric acid-best and three sucrose-best fibers. The average response profile of these 14 fibers is shown in Figure 6A. Their average spontaneous activity was 14.0 ± 2.9 impulses/5 s.

This cluster was the most broadly tuned cluster and was less uniform than the other CT clusters. Its common feature was a high sensitivity to the umami compounds, as indicated by its name, the M cluster (seven of its fibers responded best to MSG).

Generally, NaCl, LiCl and KCl elicited large responses. Amiloride did not depress the responses to either NaCl or LiCl. Since we did not record a depression of responses to salts in the whole CT nerve preparation, both summated and all single fiber data suggest absence of amiloride effects in the pig. We conclude that amiloride does not depress the response to NaCl and LiCl in this species.

Among the sweeteners, glycine, xylitol and D-tryptophane elicited moderate responses. Carbohydrates and acesulfame-K stimulated ~50% of these fibers; NHDHC, saccharin and their mixtures gave no responses.

Bitter compounds were not effective stimuli for the M fibers. Thus QHCl and denatonium elicited very small responses and then in only three fibers. No response was recorded to caffeine.

H cluster. Twelve citric acid-best fibers were included in the H cluster. Figure 6B shows the average response profile of these fibers. These fibers were the most narrowly tuned group (H = 0.51 ± 0.05) with an average spontaneous activity of 20.5 ± 5.7 impulses/5 s.

Their responses to acids were larger than to all other compounds. However, the difference was not significant for glycine. Xylitol was also an effective stimulus. Carbohydrates stimulated some of these fibers. The least effective carbohydrate stimuli were maltose, fructose and sucrose, which gave a response in ~50% of the fibers. Among the remaining sweeteners, NHDHC, saccharin, acesulfame-K, stevioside and D-tryptophane gave no response. Similarly, salts elicited no responses. With regard to bitter compounds, QHCl elicited small responses in 60% of the fibers, while caffeine and denatonium gave no responses.

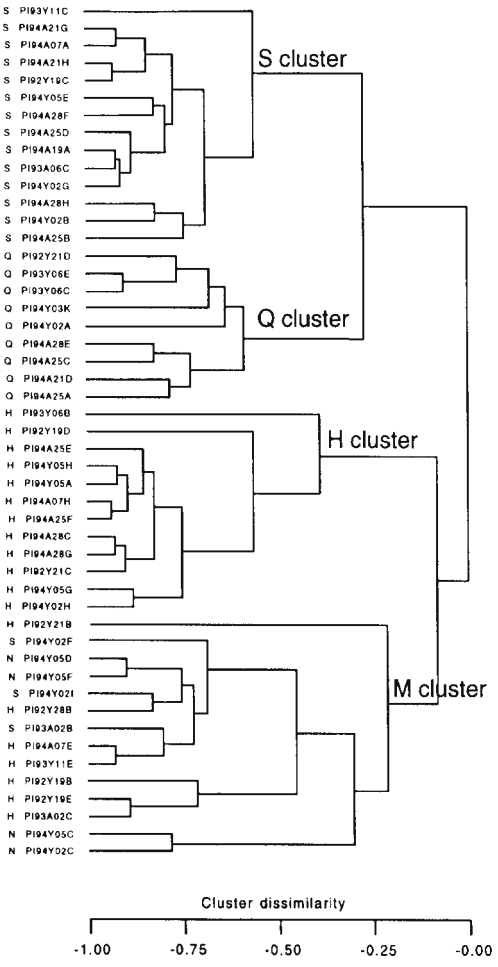

Figure 5 The result of hierarchical cluster analysis of the response profiles for 49 CT fibers. Listed on the left are response categories based on responses to the four standard stimuli: N, NaCl-best; H, citric acid-best; Q, QHCl-best; and S, sucrose-best.

Q cluster. Figure 6C shows the average response profile of the Q cluster, which consisted of nine QHCl-best fibers. Their average spontaneous activity was 4.8 ± 1.4

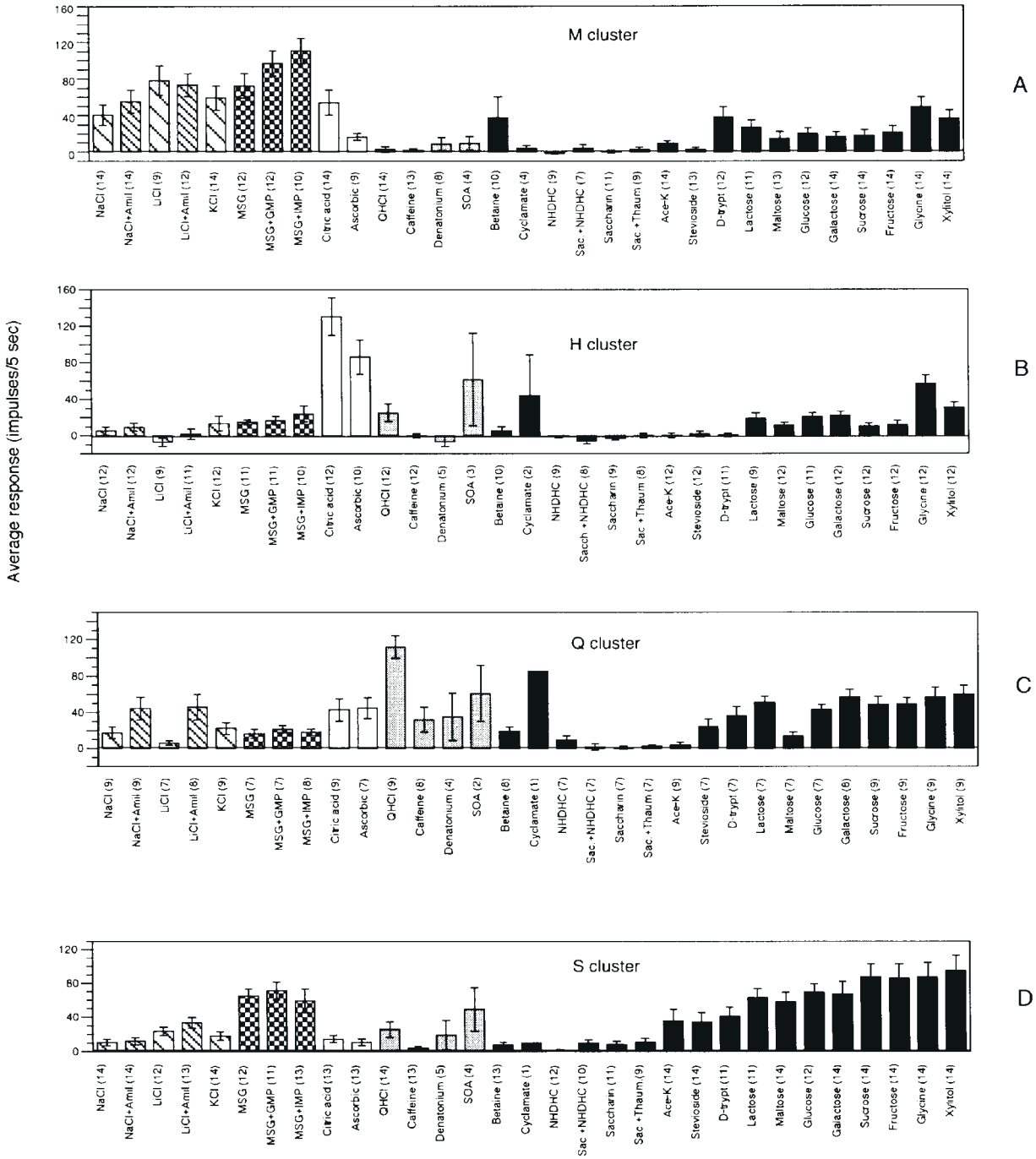

Figure 6 Average response profiles of the different clusters identified in the pig CT nerve. Error bars: SE. The number of fibers averaged for each stimulus is shown below each *x*-axis. Different patterns of columns indicate different taste qualities, as in Figure 2.

impulses/5 s. Generally the Q cluster responded to many of the compounds tested. The response to QHCl was significantly larger than the responses to all other compounds, including the other bitter stimuli. On the other hand, this group of fibers was the only one that consistently responded to caffeine, denatonium benzoate and SOA. The significantly larger response to mixtures of NaCl or LiCl with amiloride than to the salts alone indicates that amiloride itself stimulated fibers of the Q cluster.

S cluster. Figure 6D shows the average response profile of the S cluster consisting of 14 sucrose-best fibers. Their average spontaneous activity was 7.8 ± 1.5 impulses/5 s. All carbohydrates, the umami compounds, glycine and xylitol elicited good responses. These responses were significantly

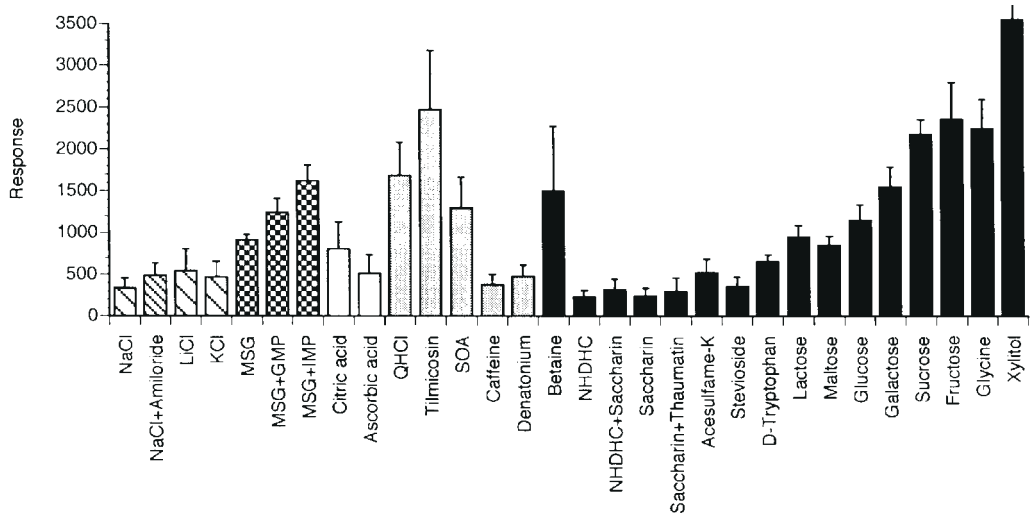

Figure 7 Averages of summated taste responses from the whole NG nerve to different stimuli. The columns represent the area of responses over the first 5 s of stimulation. Different patterns of columns indicate different taste qualities, as in Figure 2. Data from four pigs were averaged. The error bars: SE.

larger than those to salts, acids or bitter compounds. Acesulfame-K and stevioside elicited responses in 30% of the fibers. Saccharin alone or mixed with thaumatin or NHDHC stimulated some fibers, although the responses were small. NHDHC did not stimulate the S fibers. MSG alone and in mixtures elicited strong responses in all S fibers. NaCl stimulated 27%, LiCl 67% and QHCl 64% of the S fibers.

Glossopharyngeal nerve series

The whole NG response

Figure 7 presents average responses from the whole NG to all stimuli. It shows several interesting features: firstly, a large response to bitter stimuli, especially to tilmicosin, QHCl and SOA; secondly, comparatively small responses to the acids; thirdly, large responses to the sweet compounds. These responses are unusual, because in most species sweeteners elicit smaller responses in the NG than in the CT. A comparison between Figures 2 and 7 shows that the order between the sweeteners, expressed as magnitude of response, was essentially the same in the NG as in CT ($r = 0.91$). The fourth feature is that umami compounds (MSG alone and with GMP or IMP) elicited significant responses. Finally, the response to NaCl mixed with amiloride was not less than to NaCl alone.

Single fiber responses in NG

Figure 8 shows an overview of the response profiles of 31 NG fibers as a bubble chart presented in the same way as for the CT fibers. The average spontaneous activity of all fibers was 10.4 ± 1.7 impulses/5 s. The most striking findings were large responses to bitter compounds and absence of NaCl-best fibers. Table 2 shows that the sucrose-best fibers were most narrowly tuned ($H = 0.35 \pm 0.04$).

Hierarchical cluster analysis

The responses of the 31 fibers to all 30 stimuli were subjected to hierarchical cluster analysis and the result presented as a dendrogram in Figure 9. Listed on the left side is each fiber's number and response category based on its response to the four standard solutions. Our cluster analysis clearly separated four clusters: M, H, Q and S.

M cluster. Figure 10A shows the average response profile for the three fibers in this cluster. Their average spontaneous activity was 5.2 ± 3.8 impulses/5 s. These fibers responded almost exclusively to the umami compounds. All M fibers were also stimulated by sucrose and two of them by fructose and glycine. Salts, acids and bitter compounds did not stimulate these fibers.

H cluster. Figure 10B shows the average response profile for the H cluster, which consists of three citric acid-best and one sucrose-best fibers. The average spontaneous activity of these four fibers was 10.8 ± 2.3 impulses/5 s. Both citric and ascorbic acid elicited good responses. However, responses to the other stimuli were also observed. As a consequence these fibers formed the most broadly tuned group. This contrasts with our findings in the CT.

Q cluster. The Q-cluster included nine fibers with an average spontaneous activity of 15.4 ± 4.1 impulses/5 s. These fibers displayed an impressive response to QHCl, tilmicosin and SOA, while caffeine and denatonium benzoate gave smaller responses. The analysis put one sucrose-best fiber, PI94D20F, in this cluster because it responded very strongly to SOA and tilmicosin, although hardly to QHCl.

Salts were not effective stimuli for this cluster. Only two fibers responded to LiCl and KCl, and then only with small responses, while none responded to NaCl. However, a mixture of NaCl with amiloride elicited significant responses. Thus amiloride in itself was an effective stimulus

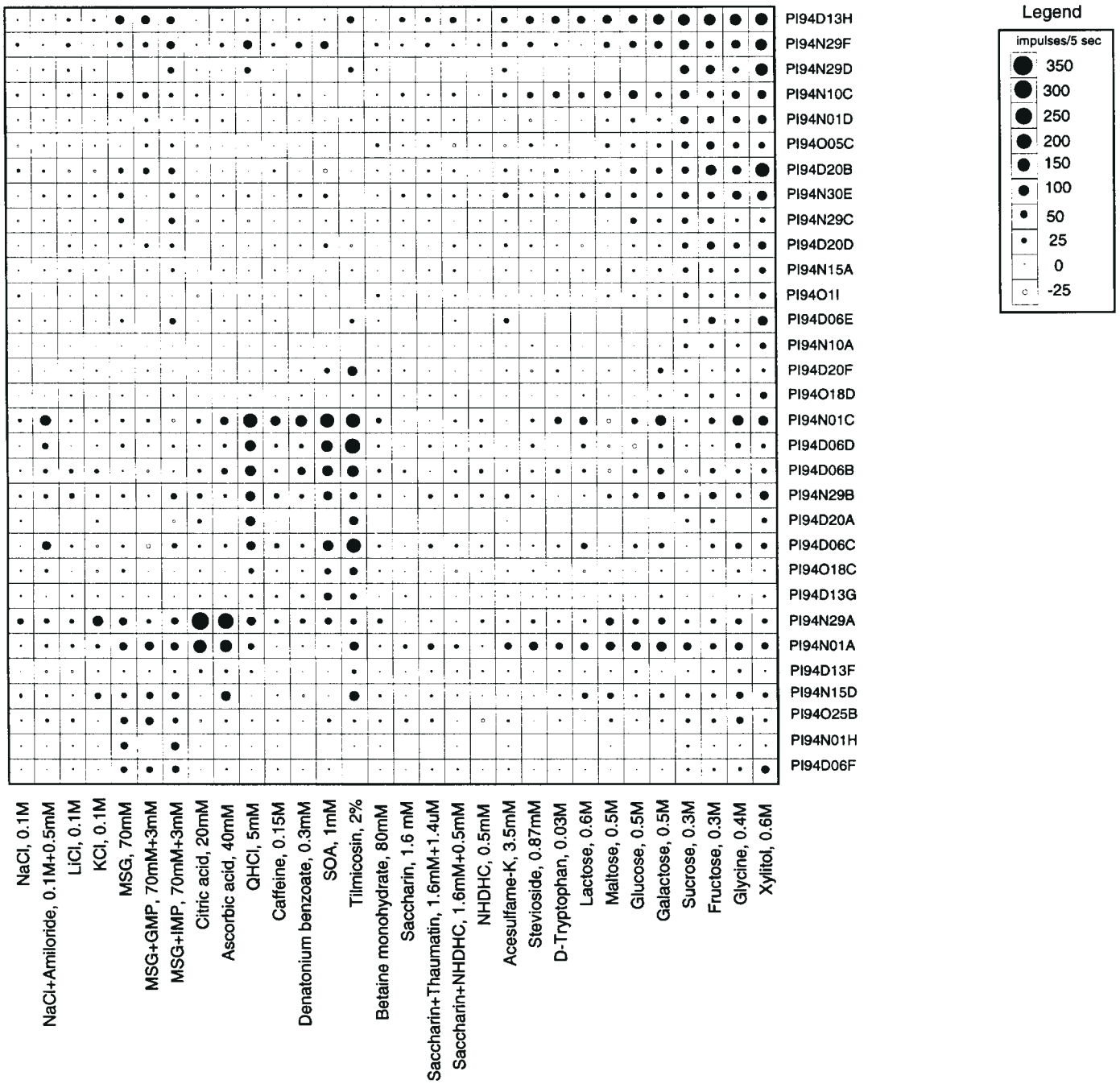

Figure 8 An overview of the response profiles of single NG fibers of the pig. The area of the circles represents impulse frequency over the first 5 s of stimulation. Open circles represent inhibition. Absence of a mark shows that data are missing. The stimuli were arranged along the *x*-axis in order of salt, umami, sour, bitter and sweet. The fibers were arranged along the *y*-axis in groups: NaCl- , citric acid-, QHCl- and sucrose-best fibers.

for the Q fibers. With regard to the sweeteners, most of the fibers responded to galactose, glycine and xylitol, while occasional responses were recorded to the other sweet compounds.

S cluster. The average response profile of the 15 fibers in the S cluster is shown in Figure 10D. Their average spontaneous activity was 8.3 ± 3.3 impulses/5 s. Sucrose, fructose, glycine and xylitol elicited responses that were significantly larger than the responses to the other stimuli. Galactose, glucose and maltose gave a response in the majority of these fibers, although it was weak. Acesulfame-K, D-tryptophan, lactose, saccharin alone and in mixtures, and stevioside gave a response in <50% of the fibers which, when present, was very weak. Interestingly, the umami

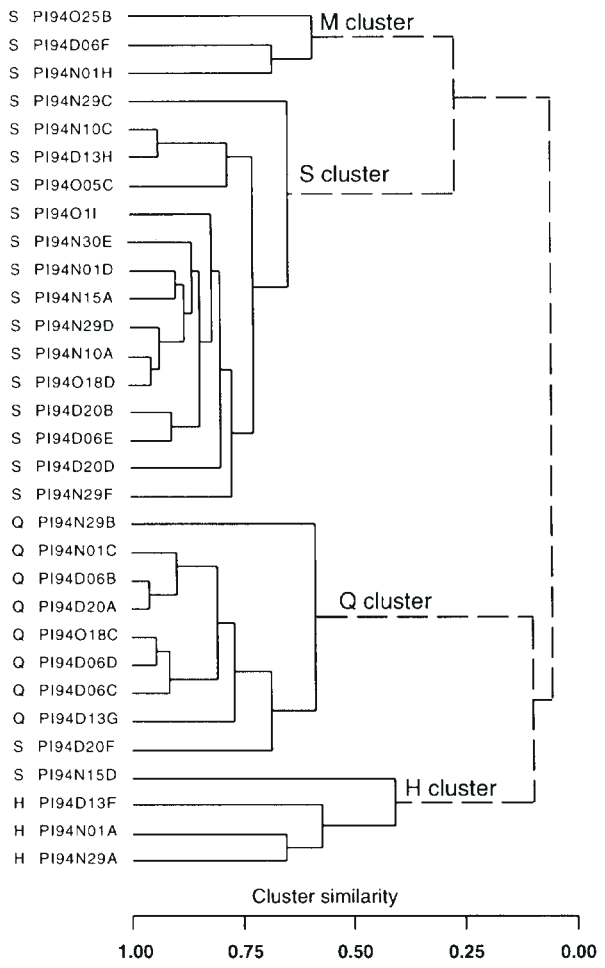

Figure 9 The result of hierarchical cluster analysis of the response profiles for 31 NG fibers. Listed on the left are the fiber's number and response category based on responses to the four standard stimuli (S, sucrose-best; Q, QHCl-best; and H, citric acid-best fibers).

compounds stimulated these fibers. The average responses to salts, acids, bitter stimuli, betaine and NHDHC did not the meet criterion of a response.

Comparison of the responses of CT and NG fibers

Since many studies use the best-stimulus classification we compared the proportion of different fibers in the CT and NG with the same method. The distribution of fibers differed significantly between the CT and NG (Chi-square = 13.09; $P = 0.004$). The acid-best fibers accounted for the largest contribution to the differences (Chi-square = 6.05; $0.01 < P < 0.05$). In the CT the majority of the fibers were acid-best fibers, while in the NG this group constituted <10% of fibers. The response to citric acid in CT acid-best fibers (122.5 ± 20.9 impulses/5 sec) did not differ significantly from the responses in the acid-best fibers of the NG (156.2 ± 96.4 impulses/5 s). The small number (four) of H fibers in the NG did not allow a firmer comparison.

The percentage of QHCl-best fibers was about the same in the NG as in the CT (25.8 and 18.8% respectively). Also their responses to QHCl did not differ significantly (NG: 91.9 ± 20.9 impulses/5 s; CT: 111.8 ± 12.1 impulses/5 s). The population of sucrose-best fibers in the NG was twice as large as in the CT (64.5 versus 33.4%), but the average response was smaller in the NG than in the CT (44.1 ± 7.4 impulses/5 s versus 76.9 ± 14.2 impulses/5 s). The small number of NaCl-best fibers in CT (8.3%) and the absence of this group in the NG seem to be special features in pigs.

Multidimensional scaling of stimulus relationship

To investigate the relationship among stimuli we performed multidimensional scaling (MDS) analysis. It computes coordinates of points in a space. The distances between these points reflect dissimilarities between corresponding objects, in our case stimuli. The result of MDS is a map showing dissimilarities between stimuli.

The MDS analysis of the data from 49 CT fibers with 23 stimuli (responses to SOA, cyclamate and six mixtures were not included) produced the result shown in Figure 11A. The MDS analysis of 31 NG fibers with 25 stimuli (again, responses to five mixtures were not included) produced the result shown in Figure 11B. The plots suggest that the sweeteners are grouped separately. Interestingly, MSG was located together with the sweeteners in both nerves.

Finally we computed the correlation matrix based on the responses of both 48 CT and 31 NG fibers to the same list of 23 stimuli. The result is displayed in Figure 11C. It shows a better separation between the bitter, salty, sour, umami and sweet compounds when information from both nerves was used.

Discussion

In most species the N and S clusters dominate the CT fiber population, while the Q cluster overshadows the other NG clusters. This contrasts with the situation in the pig, where the H cluster dominated the CT, forming 40% of the fibers, and the S cluster was most populous in the NG, comprising 48% of all fibers. In the following we discuss the clusters in order presented above: M, H, Q and S.

M cluster

The strong response in both nerves to umami compounds suggests that these compounds are powerful stimuli to the pig. Our analysis assigned 1/3 of CT fibers and 1/10 of NG fibers to the M clusters. The high specificity to umami in the NG suggests that the NG is more important than the CT for the discrimination of umami stimuli from other stimuli, especially from NaCl, since the CT also responded to NaCl. The predominant role of NG in umami discrimination corresponds well with data in mice (Ninomiya and Funakoshi, 1989) and rhesus monkeys (Hellekant *et al.*, 1997a).

Figures 2, 4, 6, 7, 8 and 10 show that amiloride did not

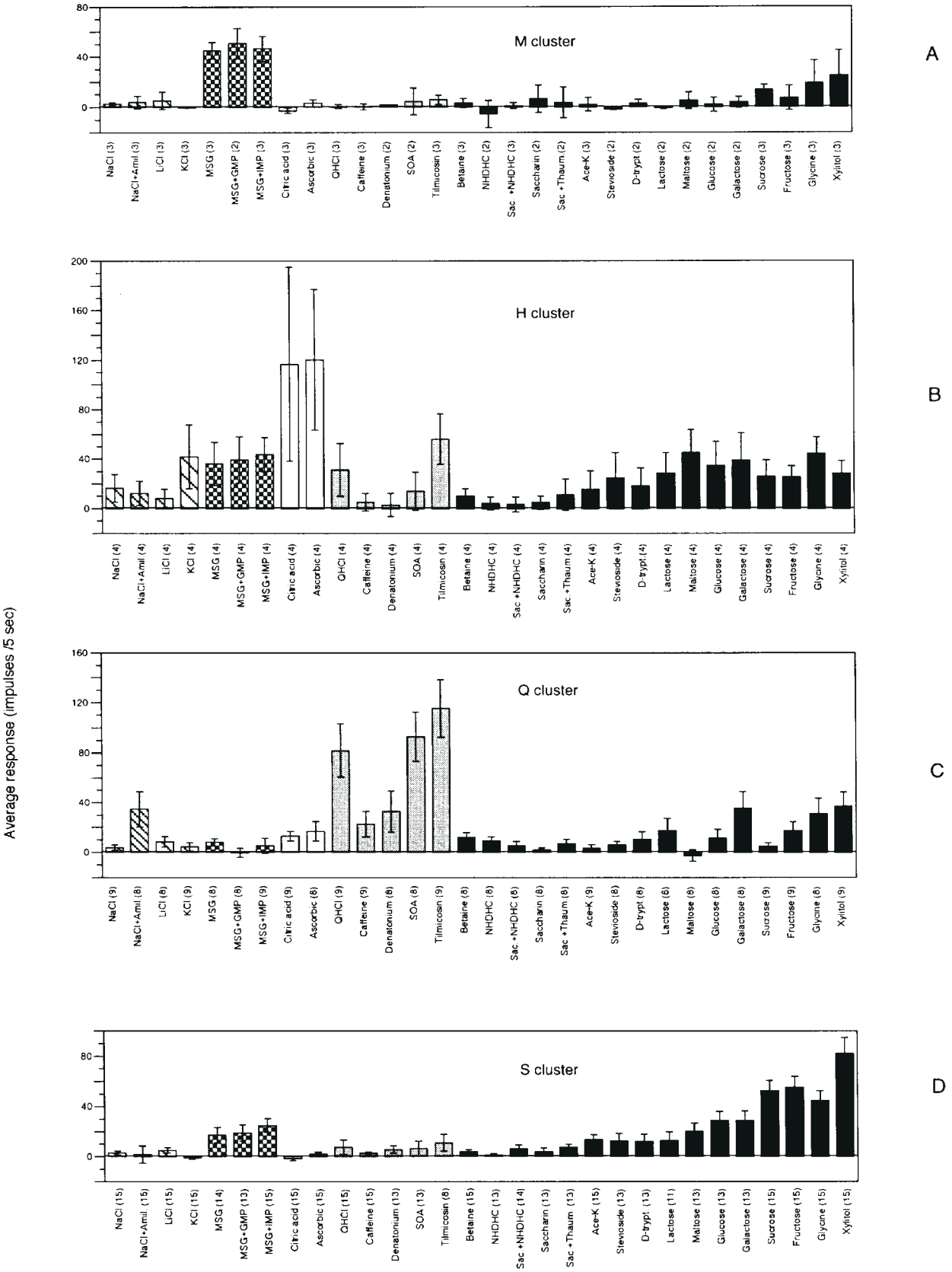

Figure 10 Average response profiles of the different clusters identified in the pig NG nerve. Error bars are SE. The number of fibers averaged for each stimulus is shown below each *x*-axis. The different patterns of the columns indicate different taste qualities, as in Figure 2.

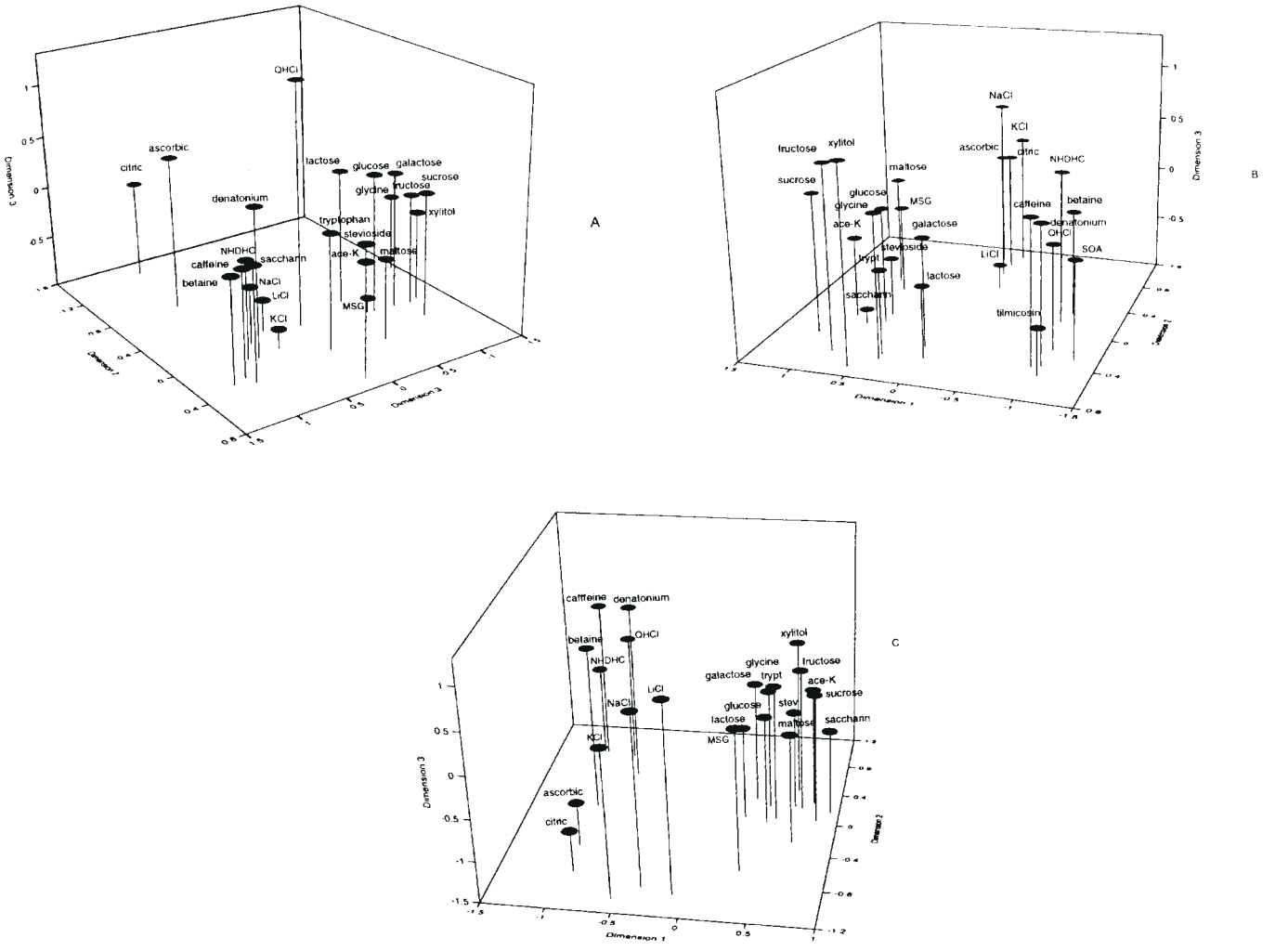

Figure 11 Three-dimensional spaces showing the location of taste stimuli, obtained from multidimensional scaling analysis. **(A)** Pearson correlation matrix of responses of 49 CT fibers was used to perform analysis. The value of stress is 0.057. **(B)** Pearson correlation matrix of responses of 31 NG fibers. The value of stress is 0.061. **(C)** The spatial representation of 24 stimuli derived from the Pearson correlation matrix of responses of 49 CT and 31 NG fibers. The value of stress is 0.062.

suppress the responses to NaCl in the whole CT and NG, or in any fiber cluster. This is interesting because in most species amiloride suppresses the responses to NaCl. For example, in the rhesus monkey, the response to NaCl in the N cluster is profoundly suppressed by amiloride (Hellekant *et al.*, 1997a). This seems to set the pig apart from most other species. It is, however, possible that the absence of amiloride effects was an age-related feature, because rats develop amiloride sensitivity postnatally over 3 weeks (Hill and Mistretta, 1990) and some of our animals were <3 weeks old. On the other hand, Figure 3 did not indicate any age-related change.

If we assume that age is not the cause, this would add the pig to the mudpuppy and two strains of mice as the few species in which amiloride has been reported not to diminish the response to NaCl (McPheeters and Roper, 1985; Ninomiya *et al.*, 1989, 1996; Gannon and Contreras, 1995). Because the umami compounds also stimulated the S cluster, we will discuss this further in that section.

H cluster

The acids elicited the largest CT responses. This shows that the pig has a high sensitivity to acids. The H cluster in the CT was most populous and its acid-best fibers most narrowly tuned. Together with the MDS results this suggests that acids create a distinct taste quality in pig. In this context it is interesting that citric and fumaric acid are used as additives in pig feed (Parker *et al.*, 1994). However, as far as the hedonic effects go, there are conflicting observations on these acids' behavioral effects. Some studies show that adding them to food caused no increase of intake (Kirchgessner and Roth, 1982), while others indicate increased intake (Falkowski and Aherne, 1984).

It should be noted that the positive behavioral effects were

reported in early weaned pigs. It is interesting that, although the difference in amplitudes was not significant, the largest acid responses were observed in the three youngest groups. These observations suggest that behavioral tests and additional nerve recordings in controlled age groups of pigs might resolve this question.

Q cluster

It is generally thought that the NG plays the main role in bitter taste. Thus, in the NG of hamsters, mice, rats and rhesus monkeys, the Q cluster constituted the largest group (Hanamori *et al.*, 1988; Ninomiya and Funakoshi, 1989; Frank, 1991; Hellekant *et al.*, 1997a). Further, in hamsters, mice and rats the responses to QHCl in QHCl-best fibers of the NG were larger than in the CT. However, in pigs there were no evident differences between the CT and the NG in the number of Q fibers, nor in their responsiveness (Figures 5 and 9). Thus it seems that pigs are different from this point of view.

We and others have earlier proposed that compounds eliciting Q fiber activity cause rejection of intake (Frank, 1991; Smith and Frank, 1993; Hellekant *et al.*, 1997b; Danilova *et al.*, 1998a,b). A comparison between pig behavioral data (Nelson and Sanregret, 1997) and our electrophysiological data for QHCl, caffeine and denatonium benzoate supports this conclusion. With regard to SOA, Figures 8 and 10C showed that 1 mM SOA elicited a good response in the Q cluster. In behavioral tests Nelson and Sanregret reported that a 10 times lower SOA concentration (0.1 mM) was rejected by 4 out of 8 pigs (Nelson and Sanregret, 1997). Finally, rejection of tilmicosin, the most powerful Q fiber stimulus here, is a major problem with its oral administration (L. Bäckstrom, personal communication). Thus it seems that activity in Q fibers accompanies rejection in pigs.

With regard to sweet taste, many investigators have observed major species differences (Beidler *et al.*, 1955, Jakinovich, 1988, p. 427; Hellekant and Danilova, 1996). This seems to be true for bitter-tasting compounds too. Denatonium benzoate is rejected by humans at nanomolar concentrations (Schiffman *et al.*, 1994) and 1 μM elicits a good CT response in chimpanzees (Hellekant *et al.*, 1997b), while millimolar concentrations have to be used in mice (Spielman *et al.*, 1996; Wong *et al.*, 1996), rats (Dahl, 1997) and guinea pigs (Nolte, 1994). Here, in the pig, 0.3 mM denatonium produced a small response. It is evident that denatonium benzoate is a less powerful tastant in pigs than in humans or apes. It thus seems that from this point of view pigs are different from higher primates.

S cluster

In agreement with the behavioral negative effects of Q fiber activity described above, we and other have observed that a response in the S fibers accompanies intake. We obtained support for this recently when evaluating the taste in hamsters and marmosets to a large number of compounds, all sweet to humans (Danilova *et al.*, 1998a,b). Our studies showed a close correlation between the S fiber response and the intake of the compound eliciting the response. This and similar findings in other species underlie the discussion below.

The carbohydrates gave large responses in the S fibers of both nerves. Behavioral studies of pigs support the conclusion on high preferences, high sensitivity and low thresholds for sugars (Lewis *et al.*, 1953; Notzold *et al.*, 1955; Fevrier, 1956; Salmon-Legagneur and Fevrier, 1956; Sachs, 1962; Spillner, 1962; Kare *et al.*, 1965; Kennedy and Baldwin, 1972; Baldwin, 1976).

Glycine and xylitol elicited strong responses in the S clusters but also stimulated Q, H and M fibers of both nerves. It is likely that in the awake pig this will cause a mixed taste reaction where positive behavioral effects of S fiber activity competes with negative effects of activity in Q fibers. Interestingly, glycine and xylitol also stimulate S and Q clusters in hamster, marmoset, rhesus monkey and chimpanzee (Hellekant *et al.*, 1997a; Danilova *et al.*, 1998a,b).

The umami compounds stimulated S fibers in addition to the M fibers above (Figure 11). In most other species responsiveness to NaCl and umami compounds correlate better than between sweeteners and umami compounds (Ninomiya and Funakoshi, 1989; Yamamoto *et al.*, 1991). For example, in macaques MSG stimulated mostly N fibers (Hellekant *et al.*, 1997a) and was not preferred in two-bottle preference tests (Pritchard and Norgren, 1991). In the pig this coupling between S fibers and umami should produce a positive hedonic response, thereby providing a rationale for the use of MSG as a feed additive in the pig.

Several of the sweeteners elicited little or no taste nerve activity. Thus alitame, aspartame, cyclamate, saccharin, super-aspartame and thaumatin elicited no or non-significant responses. The results were not different when saccharin was mixed with thaumatin or NHDHC, as shown in Figures 2, 6, 7 and 10.

Two-bottle preference tests showed that pigs are relatively indifferent to saccharin. Concentrations lower than 0.24 mM had no effects and at higher concentrations their behavior varied from rejection to preference (cf. Bradley, 1979).The electrophysiological results here provide an explanation as to why saccharin is less desirable or of no value in practical feeding.

Thaumatin is liked by catarrhine (old-world) primates. In contrast, platyrrhine (new-world) primates do not like thaumatin and do not generalize it to sucrose in conditioned taste aversion tests (Hellekant, 1976b, 1994; Hellekant and van der Wel, 1989; Glaser, 1994). In the non-primates tested, it neither elicits a taste nerve response nor is it liked (Hellekant, 1976b; Hård af Segerstad and Hellekant, 1989a,b; Danilova *et al.*, 1998a). The observation here that thaumatin gave no taste response in the CT confirms our

earlier study (Hellekant, 1976b), which showed no response in the CT of the pig. Here we recorded no response in the NG. This extends the absence of thaumatin responsiveness in the CT to the NG of pigs. It is likely that some factor other than sweetness may underlie the claim that it stimulates food intake in pigs (Higginbotham, 1994).

In summary, we found neither the same high cluster specificity nor the same level of fiber grouping according to human taste qualities as we have seen in non-human primates. This makes conclusions on preference or rejection based on single taste fiber responses in pigs more difficult than in primates. It opens up questions about what kind of taste primaries exists in pigs and to what extent pigs generalize tastes.

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