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Comparison of the responses of the chorda tympani and glossopharyngeal nerves to taste stimuli in C57BL/6J mice

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Abstract

Background: Recent progress in discernment of molecular pathways of taste transduction underscores the need for comprehensive phenotypic information for the understanding of the influence of genetic factors in taste. To obtain information that can be used as a base line for assessment of effects of genetic manipulations in mice taste, we have recorded the whole-nerve integrated responses to a wide array of taste stimuli in the chorda tympani (CT) and glossopharyngeal (NG) nerves, the two major taste nerves from the tongue.

Results: In C57BL/6J mice the responses in the two nerves were not the same. In general sweeteners gave larger responses in the CT than in the NG, while responses to bitter taste in the NG were larger. Thus the CT responses to cyanosuosan, fructose, NC00174, D-phenylalanine and sucrose at all concentrations were significantly larger than in the NG, whereas for acesulfame-K, L-proline, saccharin and SC45647 the differences were not significant. Among bitter compounds amiloride, atropine, cycloheximide, denatonium benzoate, L-phenylalanine, 6-n-propyl-2-thiouracil (PROP) and tetraethyl ammonium chloride (TEA) gave larger responses in the NG, while the responses to brucine, chloroquine, quinacrine, quinine hydrochloride (QHCl), sparteine and strychnine, known to be very bitter to humans, were not significantly larger in the NG than in the CT.

Conclusion: These data provide a comprehensive survey and comparison of the taste sensitivity of the normal C57BL/6J mouse against which the effects of manipulations of its gustatory system can be better assessed.

Background

The mouse is rapidly becoming the primary model for exploration of genetic influence on human biology. However, in order to make best use of the information now available from the sequenced genome, phenotypic information is needed. This is also the case in taste where two novel families of G protein-coupled receptors, T1Rs and T2Rs, recently have been described in both mice and human taste bud cells [1–7].

Each of these receptor families has been linked to one of the human taste qualities in such a way that the three members of the T1R family are connected to sweet taste, whereas the much larger T2R family, also labeled TRB, is associated with bitter taste. The demonstration of two non-overlapping sets of T1R and T2R expressing cells in mice suggests that sweet or bitter tastes might be encoded by activation of different subsets of taste receptor cells (TRCs) within the same taste bud [6].

There are regional differences in expression of the taste receptors on the tongue [4,6] as well as in responsiveness of CT and NG to stimuli of different taste qualities. In mice QHCl elicited larger responses in the NG than in the CT while the responses to sucrose and saccharin were larger in the CT than in the NG [8–10]. However, it is not really known if the above applies to all sweet or bitter compounds in the C57BL/6J mice. Previous recordings in CT and NG nerves have been limited to 2–3 bitter or/and sweet compounds. All other studies have presented recordings from either the CT or the NG nerves and to a limited number of compounds [8–14]. The purpose of this study is to characterize and compare the responses to a wide array of taste stimuli in the whole CT and NG nerves under the same recording circumstances, which in several experiments meant that we recorded from both nerves in the same mouse. Such information is presently scarce or missing.

Results

Figure 1 presents recordings of the whole CT and NG nerve activity in mice MO01D19 and MO01F22. In both animals the responses were obtained from both the CT and NG nerves. There are three features of particular interest in Fig. 1. First, all stimuli tested elicited a response. For some stimuli, however, there was a dramatic difference between responses elicited in the CT and NG, as was the case for denatonium benzoate, cycloheximide, NC00174. Second, within the same nerve the responses to different compounds varied in both amplitude and temporal profile. For example, the CT response to NaCl was phasic, while the responses to sucrose and the artificial sweetener NC00174 were tonic. For some compounds, for example NH₄Cl, both phasic and tonic components were present. Third, often the CT and NG responses to the same compound had different temporal profiles. The CT responses to NaCl and acesulfame-K were mostly phasic, while their responses in the NG had phasic as well as tonic components. Generally, it seems that the responses in the NG were more tonic with a less phasic component than in the CT.

The following Fig. 2,3,4,5,6,7 present the relationships between responses in the CT and NG nerves at two to four concentrations of a stimulus. In Fig. 2, 4, 6 we plotted this relationship using the integrated response, whereas in Fig. 3, 5, 7 we used the maximum amplitude of the response. In general there was no or little difference between the results of the two parameters.

Sweeteners

Figures 2 and 3 show responses to sweet stimuli in the CT and NG. All sweeteners elicited statistically significant responses in both nerves except for NC00174 and cyanosucrose in the NG. This may be interpreted as a lack of

response to these last compounds. However, they gave an NG response in two out of 8 mice. The graphs show further that for many sweeteners the responses were larger in the CT than in the NG. Thus for cyanosucrose, fructose, NC00174, D-phenylalanine and sucrose at all concentrations both the integrated response and the maximum amplitude were significantly larger in the CT than in the NG. For acesulfame-K, L-proline, saccharin and SC45647, however, the difference was not significant.

Bitter stimuli

Figures 4 and 5 show responses to bitter compounds. Based on the responses in the two nerves the stimuli can be divided into four groups. Compounds comprising the first group, cycloheximide, denatonium benzoate and PROP, elicited strong responses in the NG and no significant responses in the CT. This was true both for integrated response and maximum amplitude. However, as seen in Fig. 1, 20 mM denatonium and 10 mM PROP elicited a small phasic response in the CT in the mouse MO01F22.

The second group: amiloride, atropine and TEA stimulated both nerves, but the integrated response in the NG was significantly larger than in the CT.

The third group included compounds whose responses did not differ significantly in the two taste nerves. Several stimuli, such as brucine, chloroquine, quinacrine, QHCl, sparteine and strychnine, known to be very bitter to humans, were included in this group.

In the fourth group the relationship between the CT and NG responses to magnesium sulphate and L-phenylalanine depended on the parameter chosen, integrated response or maximum amplitude. Caffeine represents a special case. While it elicited a very small response in the NG, activity, in the CT was inhibited. As a result, the integrated response in the NG was significantly larger than in the CT. Naringine and yohimbine did not elicit significant responses.

Salts, acids and umami compounds

Figures 6 and 7 show that salts, acids and umami compounds elicited strong responses in both nerves. There were, however, differences between the CT and NG. In the CT 100 mM NaCl elicited a strong phasic response which quickly returned to the level of spontaneous activity, while in the NG it elicited both a phasic and a small tonic response. (See Fig. 1). Thus, the integrated response to 100 mM NaCl over 20 seconds of stimulation was significantly larger in the NG than in CT.

Although the integrated responses to NH₄Cl did not differ between the two nerves, the phasic component was more pronounced in the CT than in the NG (Fig. 1) and

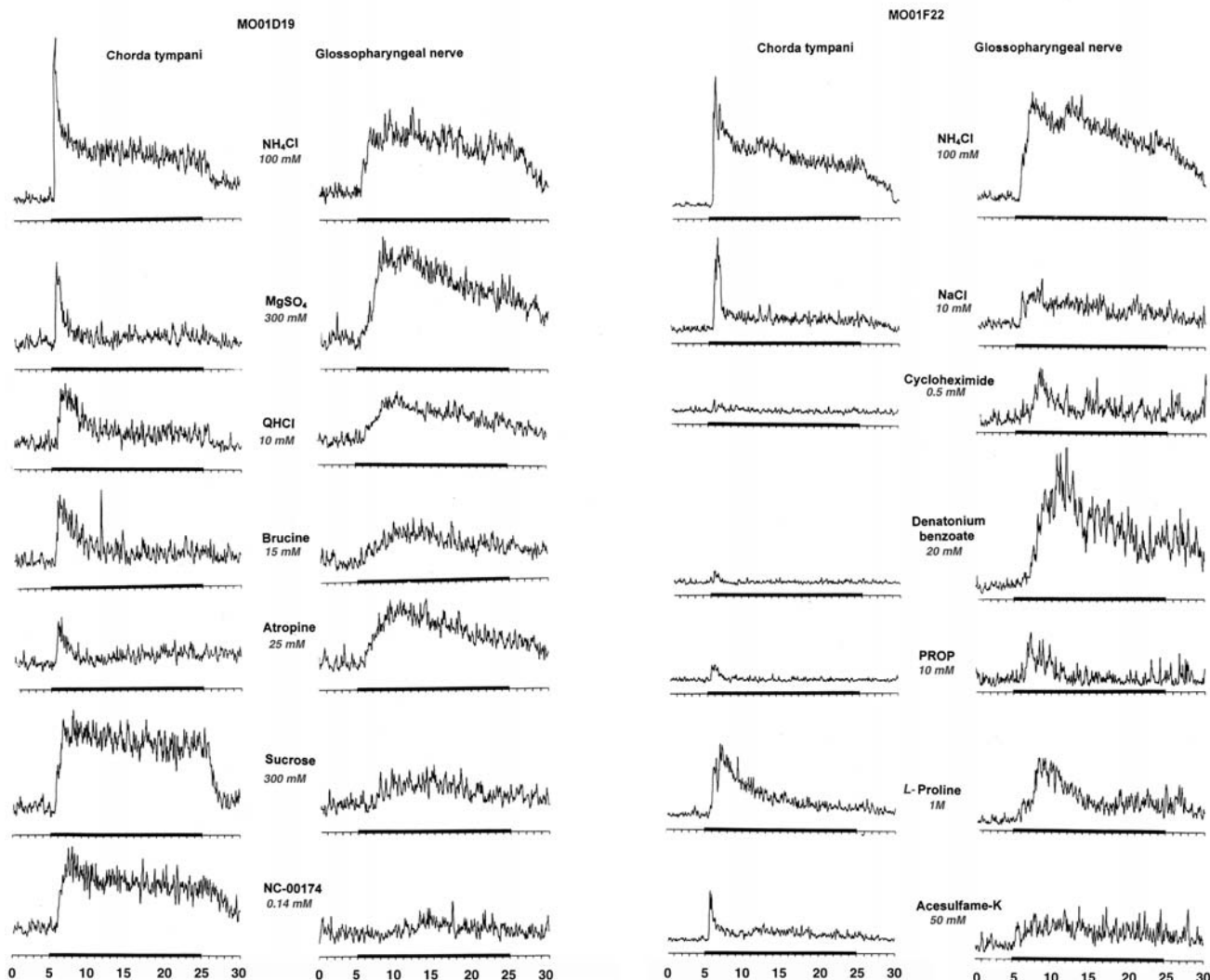


Figure 1
Summated responses from the chorda tympani and glossopharyngeal nerves during stimulation of the tongue in two C57BL/6J mice. Recordings of the whole CT and NG nerve activity in mice MO01D19 and MO01F22. In both animals the responses were obtained from both the CT and NG nerves. The glossopharyngeal nerve recordings were obtained after the chorda tympani. The horizontal axis shows the time in seconds. The thick bar at the bottom of each recording indicates the time of stimulation. Stimulation time was 20 seconds.

therefore the maximum amplitude was significantly larger in the CT than in NG.

The two umami compounds, monosodium glutamate (MSG) and disodium 5'-inosinate (IMP), performed differently. While MSG elicited a response that resembled that of NaCl, with a phasic CT response and a tonic NG response (Fig. 1), IMP gave significantly larger both phasic and tonic responses in the CT than in the NG.

Finally, the responses to citric and nicotinic acids demonstrated very similar response profiles in the two nerves with sharp onset and strong tonic responses over the stimulation.

Discussion

As mentioned in the introduction, sweet and bitter tastes are presently the focus for a number of studies using C57BL/6J mice with the intent to understand the molecular mechanisms of taste by combining genetic changes

Integrated responses of CT and NG to sweeteners in C57BL/6J mice

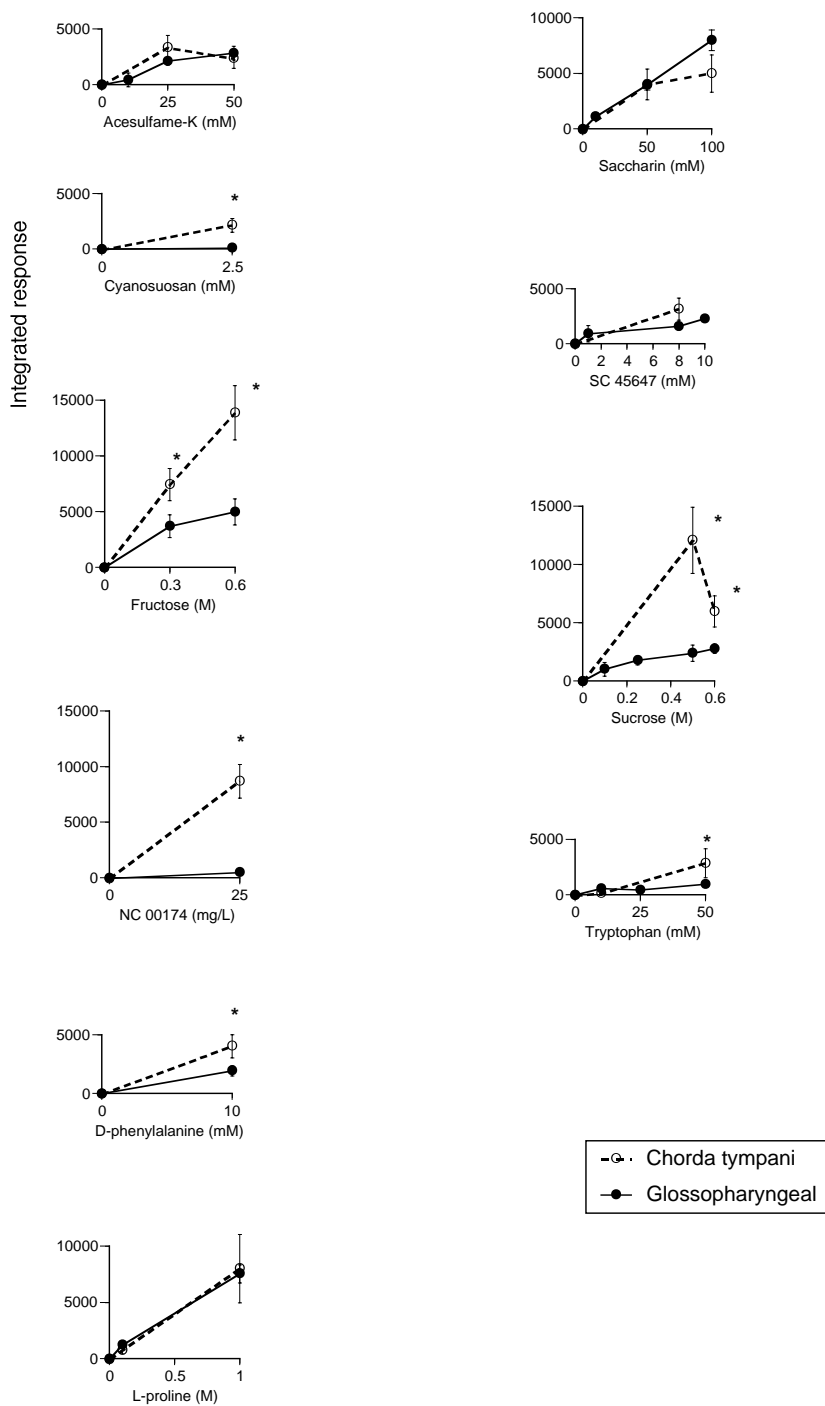


Figure 2
Chorda tympani and glossopharyngeal nerves responses to sweeteners. Comparison of the chorda tympani (open circles, dashed line) and glossopharyngeal nerves (black circles, solid line) integrated responses to sweeteners. Error bars are SE. Asterisks indicate significant (p < 0.05) difference between responses of the two nerves.

Maximum amplitude of responses of CT and NG to sweeteners in C57BL/6J mice

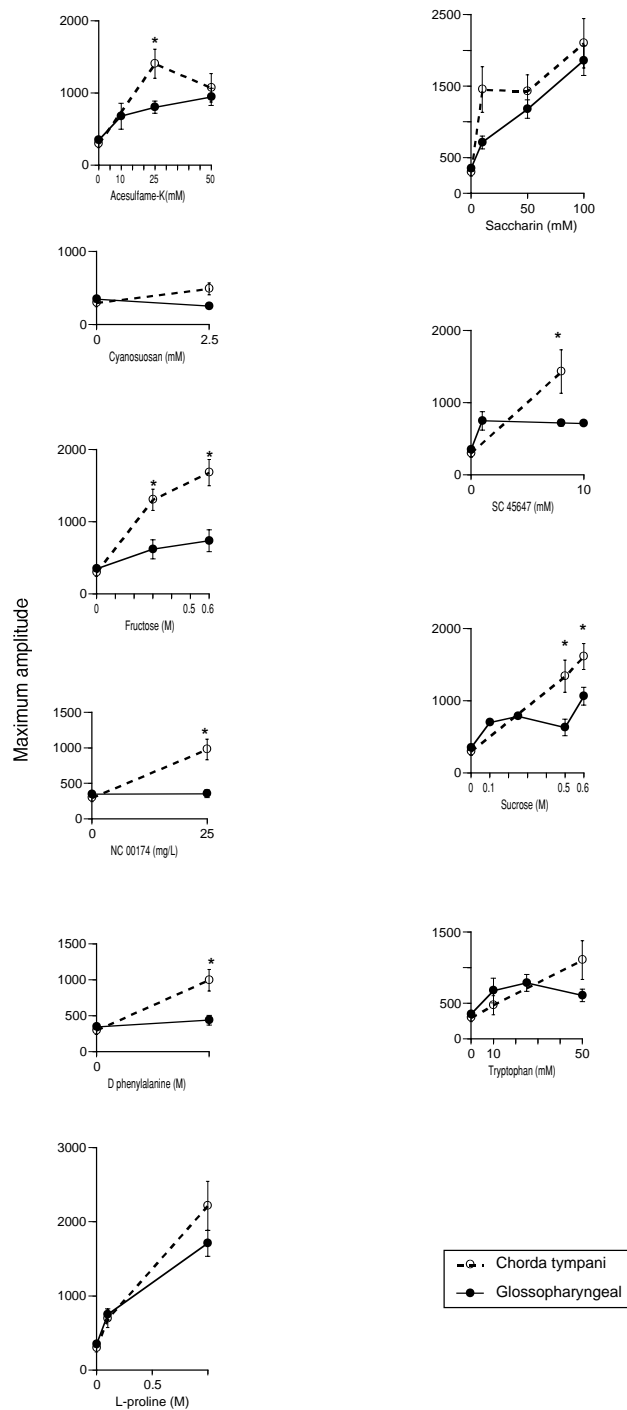


Figure 3
Chorda tympani and glossopharyngeal nerves responses to sweeteners. Comparison of the chorda tympani (open circles, dashed line) and glossopharyngeal nerves (black circles, solid line) responses to sweeteners. Maximum amplitude was used as a parameter. Error bars are SE. Asterisks indicate significant ($p < 0.05$) difference between responses of the two nerves.

Integrated responses of CT and NG to bitter stimuli in C57BL/6J mice

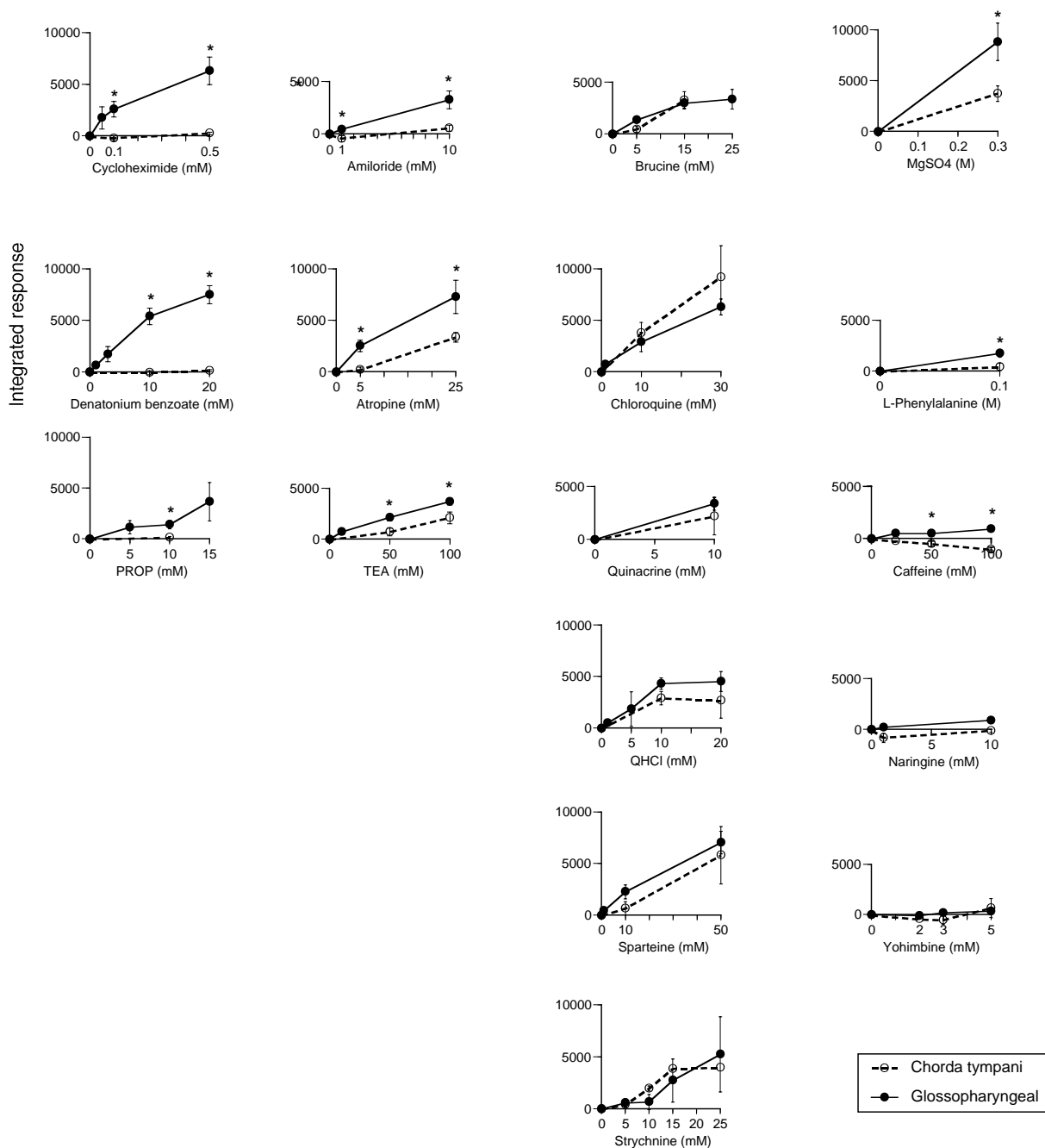


Figure 4
Chorda tympani and glossopharyngeal nerves responses to bitter compounds. Comparison of the chorda tympani (open circles, dashed line) and glossopharyngeal nerves (black circles, solid line) integrated responses to bitter stimuli. Error bars are SE. Asterisks indicate significant ($p < 0.05$) difference between responses of the two nerves.

Maximum amplitude of responses to bitter stimuli in C57BL/6J mice

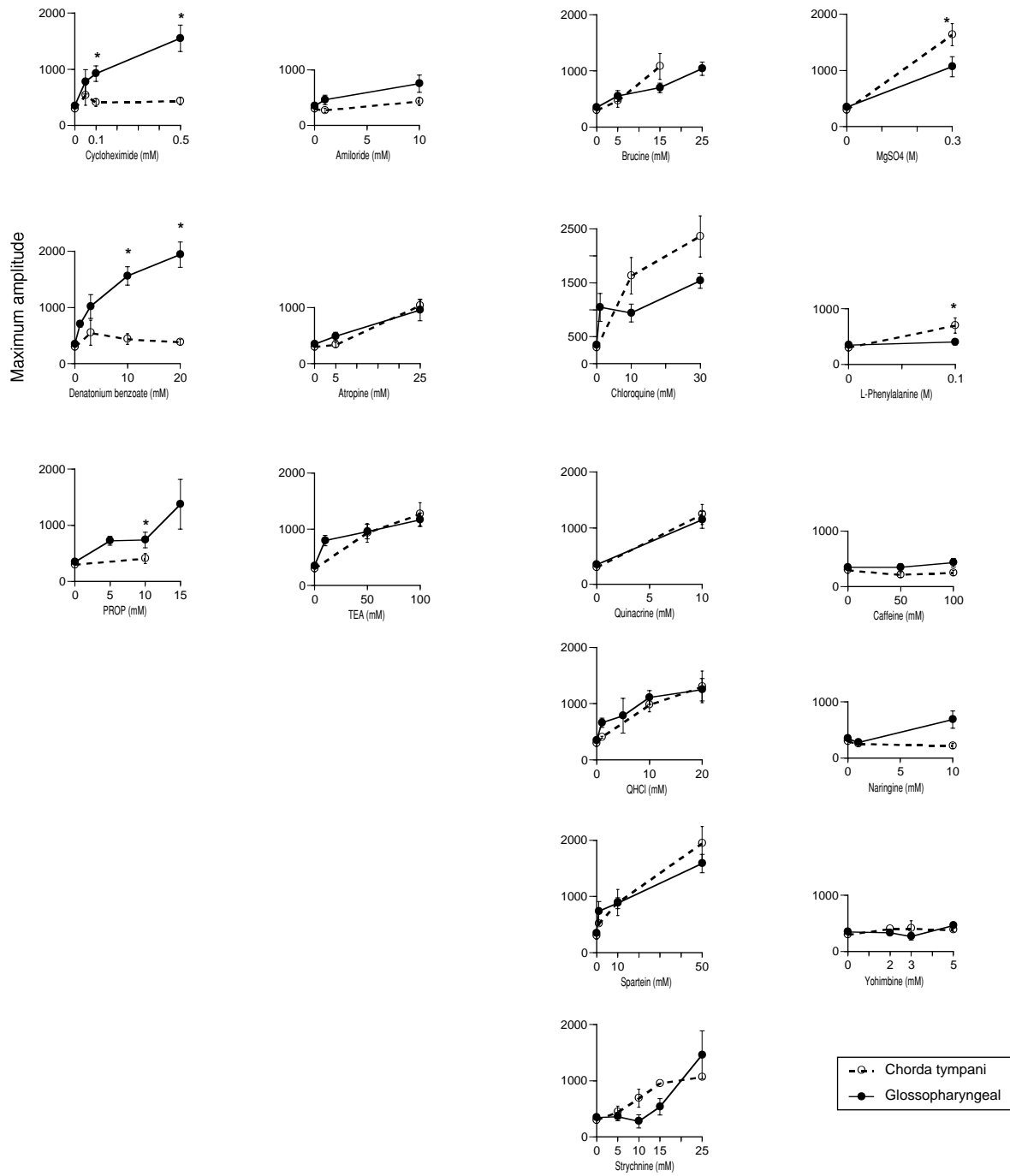


Figure 5
Chorda tympani and glossopharyngeal nerves responses to bitter compounds. Comparison of the chorda tympani (open circles, dashed line) and glossopharyngeal nerves (black circles, solid line) responses to bitter stimuli. Maximum amplitude was used as a parameter. Error bars are SE. Asterisks indicate significant ($p < 0.05$) difference between responses of the two nerves.

Integrated responses of CT and NG to salts and acids in C57BL/6 mice

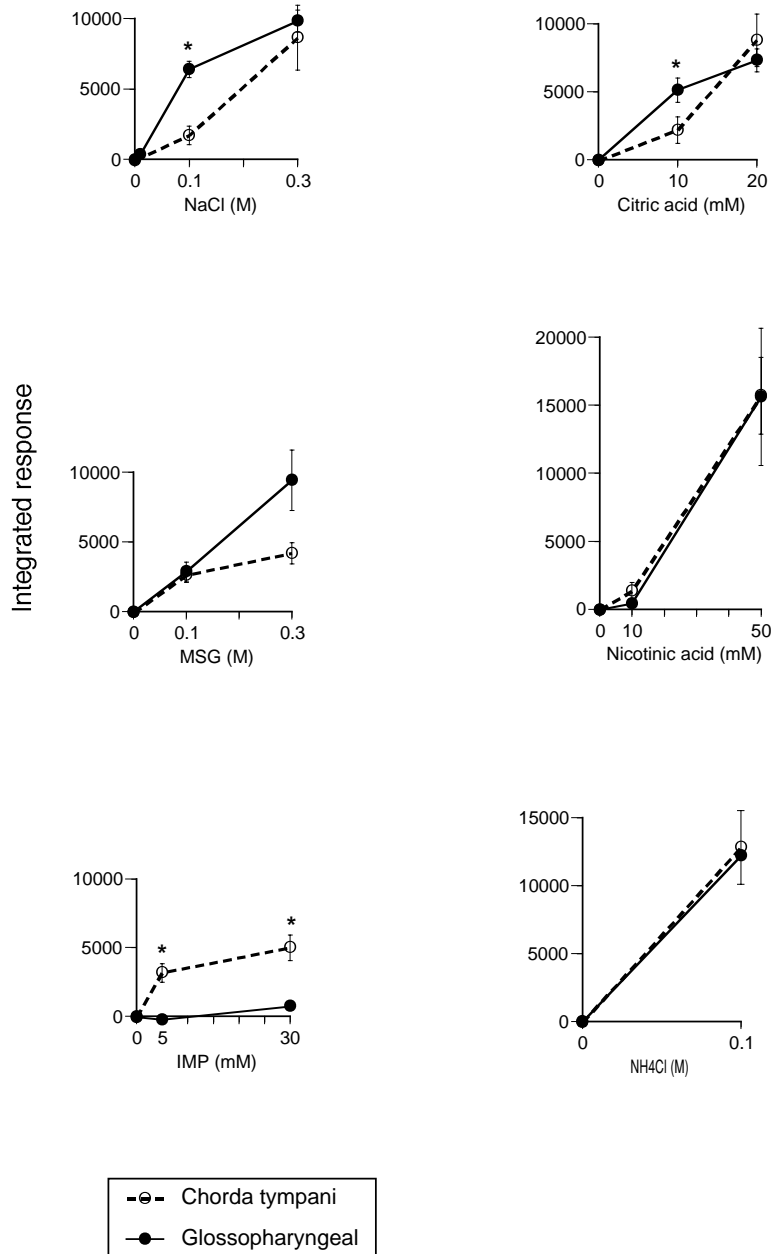


Figure 6
Chorda tympani and glossopharyngeal nerves responses to salts, acids and umami compounds. Comparison of the chorda tympani (open circles, dashed line) and glossopharyngeal nerves (black circles, solid line) integrated responses to salts and acids. Error bars are SE. Asterisks indicate significant ($p < 0.05$) difference between responses of the two nerves.

with phenotypical observations. The identification of intracellular transduction mechanisms, such as gustducin, [15,16] and more recently the sweet T1R and bitter T2R receptor families [4,6,17], offer great possibilities to further our understanding of taste. However, it is evident that parallel information on how the normal C57BL/6J mouse tastes sweet and bitter compounds is needed to fully utilize these possibilities. For example, without the information that aspartame has no taste, or that SC45647 and its analog NC00174 (compound GA1 and GA2 in [6]) are very attractive to C57BL/6J mice, it is impossible to use these compounds as ligands to assess the impact of a genetic manipulation for sweet and bitter taste. It is against this background we undertook this study, to provide basic data on the taste of a number of bitter and sweet compounds with potential use as representatives of sweet and bitter ligands for genetic and molecular biology studies.

In the following we will discuss distribution of sweet and bitter tastes in the two nerves, the choice of the compounds used and two methodological questions: how we measure taste nerve responses and should the responses be expressed in some kind of a standard, for example, as a percentage of the response to NH₄Cl?

The results presented above show that generally sweet compounds gave the larger response in the CT, while the response to bitter compounds was larger in the NG. This is summarized in Fig. 8, which shows the overall difference between the CT and NG responses for most of the compounds used in this study. To generate Fig. 8, we subtracted the integrated responses in the NG from the CT responses. In cases where several concentrations were used we averaged the responses over several concentrations. The values for all bitter compounds were negative which means that integrated responses in the NG were larger than in the CT. In contrast, for all sweeteners besides saccharin the values were positive which means that integrated responses in the CT were larger than in the NG. This graph, therefore, shows the overall trend without showing whether the differences were statistically significant. In regard to saccharin, the response in the NG was larger than in the CT at high concentration, 100 mM, but not at the lower concentrations, 10 or 50 mM. These data correlate with behavioral experiments in C57BL/6J mice. They preferred saccharin at concentrations up to 85 mM and rejected saccharin at higher concentrations [18]. In humans a high concentration of saccharin has a pronounced bitter and sweet taste. This may also be the case in mice. Together these might indicate that saccharin stimulates both sweet and bitter receptors but that the affinity to T2Rs is much lower. Thus, the "bitter" component of saccharin can be seen only at high concentrations and is reflected in a more prominent NG response.

Previously in all strains tested (LP, BDP, DBA, RF, BALB, Slc:ICR, SWR) bitter compounds, quinine (hydrochloride or sulfate) or sucrose octaacetate (only in SWR strain), elicited larger responses in the NG than in the CT [8–12,14,19]. In contrast, responses to sucrose and saccharin were larger in the CT than in the NG. These authors concluded that the response to sweet compounds dominates the front of the tongue, while bitter taste is prevalent on the back. However, in these electrophysiological studies only 2–3 bitter or sweet compounds were tested. Here we present a study of the CT and NG responses to 17 bitter and 10 sweet compounds and found a similar distribution of sensitivities in the two major taste nerves. This probably reflects the distribution of the taste transduction mechanisms in the mouse tongue.

The distribution of the responses to bitter and sweet stimuli in the nerves resembles the results observed also in some other species. In rhesus monkeys we found a larger proportion of Q fibers than S fibers in the NG, while the CT contained a larger proportion of S fibers than Q fibers [20]. Similar regional differences in taste sensitivity or cluster distribution between the back and anterior parts of the tongue have been documented in rat, hamster and pig [21–25].

In the past such topographical distribution of different taste qualities was interpreted in a way that the CT was considered to play the more important role in sweet taste while the NG in bitter taste. However, the size of electrophysiological responses does not necessarily reveal the relative importance of the taste nerves in taste-mediated behavior. Recent behavioral experiments in rats stress the prominence of the CT over the NG for the bitter taste [26–28]. We have found that mice with bilateral CT cut have a significantly impaired ability to discriminate water from the bitter stimulus denatonium benzoate in two bottle preference (TBP) tests, while mice with bilateral NG cut showed no difference from the sham surgery group (own unpublished observations). This suggests that a small phasic response in the CT to denatonium benzoate might be at least as important as the large NG response for the decisions to be made in TBP tests.

The choice of compounds (Table 1) was based on a combination of human taste experiences, behavioral and electrophysiological experiments in C57BL/6J mice and molecular data. The sweeteners we used here are preferred by mice in TBP [[18,29,30], own unpublished data on NC00174 and cyanosuosan]. Similarly behavioral data showed that the compounds we characterize as bitter were shunned by mice [[31–35], own unpublished data on sparteine, chloroquine and TEA]. Additionally, fructose, glucose, D-phenylalanine, L-proline, saccharin, sucrose

Table 1: List of solutions and concentrations (mM) used in three series of experiments with C57BL/6J mice

Stimulus	1st series (only NG)	2nd series (CT, NG)	3rd series (CT, NG)
NaCl	100 (12)	10 (0,2); 100 (9,8); 300 (5,6)	100 (6,8)
MSG	-	-	100 (6,8); 300 (6,8)
IMP	-	-	5 (6,8); 30 (5,8)
Citric acid	20 (12)	10 (9,7); 20 (9,8)	20 (5,8)
Nicotinic acid	-	-	10 (6,8); 50 (5,7)
QHCl	1 (4); 10 (12); 20 (2)	1 (2,2); 10 (8,6); 20 (8,8)	10 (6,8)
Amiloride	1 (4); 10 (4)	-	1 (6,8); 10 (6,8)
Atropine	-	-	5 (6,8); 25 (6,7)
Brucine	5 (4); 15 (2); 25 (4)	15 (5,3)	5 (5,8); 15 (6,8)
Caffeine	20 (2); 50 (2); 100 (2)	-	20 (1,0); 50 (3,5); 100 (6,8)
Chloroquine	1 (3); 10 (7); 30 (3)	10 (7,4); 30 (7,4)	-
Cycloheximide	0.05 (2); 0.1 (5); 0.5 (4)	0.05 (2,2); 0.1 (9,7); 0.5 (9,7)	-
Denatonium	1 (2); 3 (3); 10 (8); 20 (3)	3 (2); 10 (9,7); 20 (9,8)	-
MgSO4	-	-	0.3 (5,8)
Naringine	10 (2)	-	1 (4,6); 10 (2,2)
L-phenylalanine	-	-	100 (5,8)
PROP	5 (3); 10 (2); 15 (2)	10 (5,3)	-
Quinacrine	10 (5)	10 (7,7)	-
Sparteine	10 (7); 50 (4)	10 (9,7); 50 (9,7)	-
Strychnine	-	-	5 (6,8); 10 (2,3); 15 (1,2)
TEA	10 (3); 50 (5); 100 (3)	50 (7,6); 100 (8,5)	-
Yohimbine	-	-	2 (1,1); 3 (3,5); 5 (2,2)
Sucrose	500 (2); 600 (10)	600 (9,8)	500 (5,8); 600 (1,0)
Acesulfame-K	1 (2); 10 (2); 25 (2)	25 (9,6); 50 (7,5)	-
Cyanosuosan	-	-	2.5 (5,5)
Fructose	-	-	300 (6,8); 600 (6,8)
NC00174	-	-	0.14 (6,8)
D-phenylalanine	-	-	100 (6,8)
L-proline	100 (1), 1000 (3)	100 (9,6), 1000 (9,7)	-
Saccharin	10 (3); 50 (3); 100 (5)	10 (4,4); 50 (9,6); 100 (9,6)	-
SC 45647	1 (2); 10 (2)	8 (7,5)	-
D-tryptophan	10 (2); 25 (3); 50 (3)	10 (7,5); 50 (7,5)	-
NH4Cl	100 (12)	100 (9,8)	100 (6,8)

CT – chorda tympani, NG – glossopharyngeal nerve, MSG – monosodium glutamate, IMP – disodium 5'-inosinate, QHCl – quinine hydrochloride, PROP – 6-n-propyl-2-thiouracil, TEA – tetraethyl ammonium chloride. Concentrations are in mM. All chemicals were purchased from Sigma Chemical Co., St Louis, MO besides acesulfame-K (gift of Hoechst), cyanosuosan (gift of C.Nofre), NC00174 and SC45647 (gift of Nutrasweet), MSG and IMP (gift of Adjinamoto). Figures in parentheses show number of animals tested for each concentration of a stimulus. In the first series it shows the number of recordings from the NG. In second and third series the first figure is the number of CT recordings and second figure is the number of NG recordings.

and D-tryptophan cross-generalized in conditioned taste aversion experiments [29]. These results suggest that these compounds taste similar to C57BL/6J mice.

Furthermore recordings from single CT fibers in C57BL/6J followed by hierarchical cluster analysis have revealed a narrowly tuned cluster of "sweet" fibers predominantly responding to fructose, glucose, D-phenylalanine, saccharin, sucrose and D-tryptophan [36,37].

Finally, recent molecular data suggest that the compounds we assumed were sweet or bitter have a taste consistent with this conclusion. Rat T1R2/T1R3 recognized sweet-tasting molecules as diverse as acesulfame-K, dulcin,

NC00174 (GA2), saccharin, SC45647 (GA1) and sucrose [6], and mT2R-8 and-5 responded to the bitter compounds denatonium, 6-n-propyl-2-thiouracil and cycloheximide respectively [17]. Thus, although it is clear that the murine taste world in many ways differs from that of humans, the taste of the compounds used here to the mouse seems to be similar to their taste in human.

There is, however, some discrepancy between the pattern of T1R expression and physiological data in mice. Thus T1R2 and T1R3 were coexpressed in circumvallate and foliate taste buds but not in fungiform papillae [6], while sweeteners elicited stronger responses in the CT than in the NG. We have also found that mice in TBP tests with bi-

Maximum amplitude of responses of CT and NG to salts and acids in C57BL/6 mice

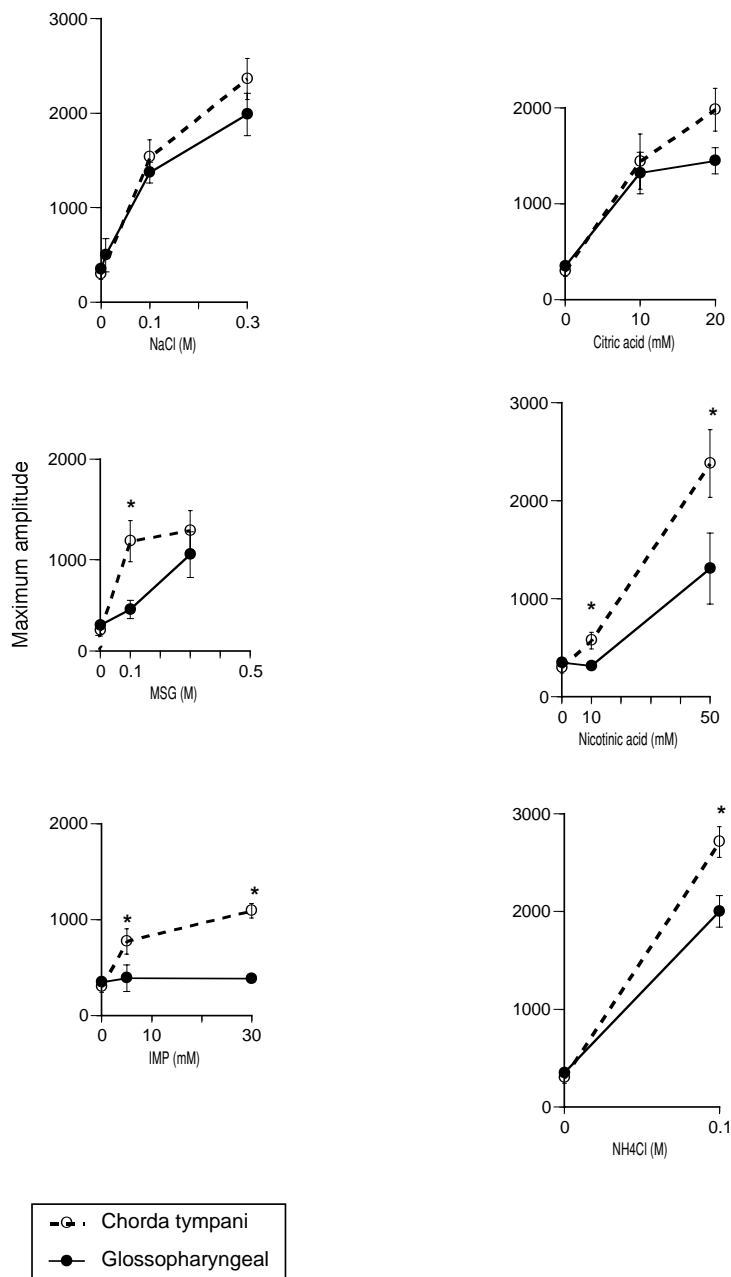


Figure 7
Chorda tympani and glossopharyngeal nerves responses to salts, acids and umami compounds. Comparison of the chorda tympani (open circles, dashed line) and glossopharyngeal nerves (black circles, solid line) responses to salts and acids. Maximum amplitude (B) was used as a parameter. Error bars are SE. Asterisks indicate significant ($p < 0.05$) difference between responses of the two nerves

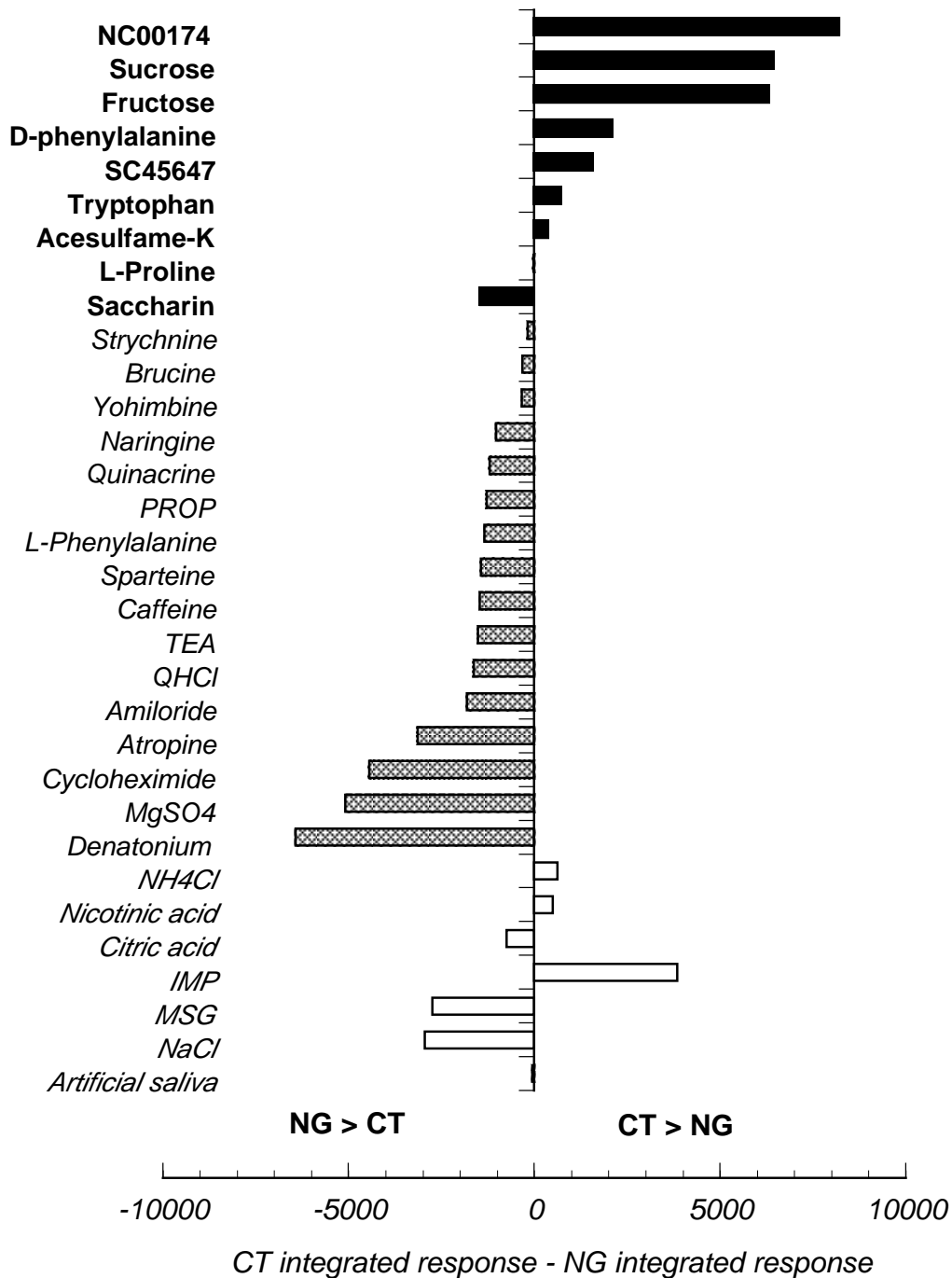


Figure 8
Difference between chorda tympani and glossopharyngeal responses. Integrated response was used as a measure of responses. Different taste qualities of stimuli (for humans) are coded by different pattern in the columns and different font in the names. Open columns and italicized names indicate salts, umami compounds and acids; cross-hatched, bitter compounds; and black columns and bold font, sweeteners. In cases when several concentrations of a compound were tested the responses were averaged and then subjected to the comparison. Generally, responses to sweeteners were larger in the CT and responses to bitter compounds were larger in the NG.

lateral CT cut have a significantly diminished preference for fructose and NC 00174, while mice with bilateral NG cut showed no difference from the sham surgery group (own unpublished observations). This would not be the case if there were no sweet receptors in the area innervated by the CT. It is possible that there is another sweet receptor in fungiform taste buds, as has been suggested [6].

Here we used two parameters to characterize the summated responses. One was the amplitude of the response. It is the method used in most studies. However, which part of the amplitude that is used varies. We have used the maximum amplitude regardless of when it occurs. Some investigators used the maximum amplitude of the "slow phase" of the whole response [12], others measured the amplitude after a particular time, for example 20 sec [10,13]. These methods, however, do not take into consideration differences in the temporal profiles, which vary considerably as shown in Fig. 1. Therefore, measuring the amplitude at two different intervals, for example within the first 2 sec and at 10 sec, gives a better representation of the response [38,39].

It is also evident that using maximum amplitude as a measure of response can be problematic when the spontaneous activity in the taste nerve varies considerably. As a consequence, a small phasic response may pass unnoticed. Alternatively, spontaneous variations of nerve activity during stimulation may result in a positive measure although there was no response. This can be seen in Fig. 3, 5 and 7 for zero stimulus concentration.

The other parameter we used was the integrated response which also suffers some shortcomings. For example, the stimulus may only elicit a short phasic response which then will be overlooked if only the integrated response is used, as for denatonium benzoate in Fig. 1. The use of both parameters best describes the responses.

Another question is if the difference in temporal profile was caused by some intrinsic factor in the transduction mechanism or the result of the location of the taste buds. We think that the difference in accessibility to the taste buds innervated by CT and NG is probably not the whole explanation, because the NG responses to NH₄Cl, NaCl and citric acid, displayed a similar phasic component as their responses in the CT. Thus it is likely that the difference in temporal profiles may be related to different mechanisms of taste transduction.

The last issue to be discussed is if the data should be normalized, that is, expressed in relation to a standard? NH₄Cl is commonly used as standard in mice and rats, although responses to NaCl, sucrose as well as an average of the four standard stimuli (NaCl, acid, QHCl and sucrose)

have been used [10,39]. Normalization might be necessary when combining data from a homogeneous group of animals with large individual variation in nerve activity or to compare data between different groups (strains) of animals with large variations in nerve activity between the groups. Another reason is if one nerve is systematically more active than another. Here there was no difference between the integrated responses to NH₄Cl in CT and NG. Consequently normalization to NH₄Cl would not have changed our results.

A possible disadvantage of using non-normalized data is that the results can be affected by animal to animal or nerve to nerve variations or by other external factors, such as anesthesia, signal amplification, etc. In this study we kept the level of amplification constant between recordings of the two nerves in the same mouse and between the mice. Furthermore, the level of activity during rinsing with artificial saliva did not differ between the nerves. We conclude that there was no need to normalize the data presented in this study.

Methods

Animals and surgery

Thirty male mice of the C57BL/6J strain from the Jackson Laboratory (Bar Harbor, ME) were used in three series of experiments. The mice were 9–12 weeks old and weighed 20–29 g at the time of recordings. Data were obtained in three different series. In the first series we recorded only from NG. In the second and third series we recorded from both CT and NG. In Table 1 the figures in the parentheses show the number of animals tested with each stimulus in each series. The first figure within the parenthesis shows the number of CT recordings and the second figure the number of NG recordings. Thus, for example, the top line in the third column shows that we recorded the response to 100 mM NaCl in 6 CT and 8 NG nerves.

The mouse was anesthetized with an i.m. injection of a mixture of 1.75 mg/ml ketamine and 1.75 mg/ml xylazine in saline (5 µl/g b.w.) and then intubated. The anesthesia was maintained with 0.4–0.6% isoflurane. Body temperature and heart rate were continuously monitored. The CT nerve was dissected free from its junction with lingual nerve to the tympanic bulla. The NG was accessed through the same incision as the CT and cut near to its exit from the posterior lacerated foramen [40]. In 13 mice we recorded first the responses of the CT during stimulation of the tip of the tongue. Then we dissected and recorded from the NG of the same animal. The order between the CT and NG nerve recordings could not be changed, because the tip of the tongue was damaged during the NG recording when we stretched the tongue to insure that the foliate and vallate taste buds were stimulated.

Stimulation

The stimuli were delivered to the tongue with an open flow system controlled by a computer. It delivered the solutions at given intervals, over a preset time, under constant flow and temperature (33°C) [41]. Each stimulation lasted for 20 s with 50 s rinsing time between stimulations. Table 1 lists the stimuli and their concentrations used in each of three series of experiments. All compounds were dissolved in artificial saliva. Between stimulations the tongue was rinsed with artificial saliva, which contained 2 mM NaCl, 5 mM KCl, 3 mM NaHCO₃, 3 mM KHCO₃, 0.25 mM CaCl₂, 0.25 mM MgCl₂, 0.12 mM K₂HPO₄, 0.12 mM KH₂PO₄, 1.8 mM HCl for pH 7. Because the precise content of mouse saliva is not published, we decided to use twice-diluted formula of the artificial saliva we have used in other species (monkey, pig, rat) [20,25,41]. It maintained the taste buds in good condition and gave us stable and reproducible nerve recordings for many hours. Artificial saliva was also used as a stimulus in itself. Thus the nerve activity during 20 s of stimulation with saliva was considered as a response to 0 concentration of any other stimulus.

Care was exercised to make sure that the tongue was optimally stimulated. This was easy during the CT experiments when the flow was easily visible. During the NG recordings, we checked with a surgical microscope that the flow ran over the vallate and foliate papillae. A further test was to see whether the summated response had a sharp onset to NH₄Cl, NaCl, and the acids, because the sharp onset revealed if the taste buds on the back of the tongue were adequately stimulated and assured that the possible absence of a phasic component of the responses to other stimuli was related to the property of the stimulus and not an artifact.

Recording and analysis

Nerve impulses were recorded with a custom-made amplifier, monitored over a loudspeaker and an oscilloscope, and fed into a recorder (Gould TA-11). For the whole nerve recordings the nerve impulses were processed by a smoothed absolute value circuit integrator [41] and changed to a DC potential whose amplitude was related to the nerve impulse frequency, here called the integrated response. This signal and a code related to the tastant on the tongue were fed via a DAS-Keithley interface to an IBM computer. Its program sampled the summated response before, during and after stimulation and displayed it on a monitor.

To characterize the whole nerve response we used two measures. First, the integrated response during stimulation calculated as the surface area under the trace and expressed in arbitrary units. The integrated response was obtained by deducting the area of spontaneous nerve

activity (preceding the stimulation) from that during stimulation. Thus it reflects the level of activity during 20 seconds of stimulation time. Our second response measure was the maximum amplitude of a response within 20 seconds of stimulation.

To assess whether the CT or NG responses to the stimuli were different from the response to 0 concentration we used two-tailed t-tests. For compounds tested in more than one series (Table 1) responses from different series were pooled.

Then integrated and maximum responses were analyzed with separate ANOVAs. Because we found no correlation between the integrated responses in the CT and NG in the same animals (data not shown), we treated these responses as independent measures. Thus we pooled data from mice with recordings from only one nerve and data from mice with recordings from two nerves. Responses were evaluated with two-way ANOVA with nerve as a between-groups factor and concentration as a within-group factor. Post hoc comparisons of stimuli using Student-Newman-Keuls test were performed to check differences between CT and NG responses at specific concentrations. For stimuli presented at single concentrations the differences were assessed with two-tailed t-tests. For all statistical tests $p < 0.05$ was considered significant.

Authors' contributions

The authors contributed equally to this paper.

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