Chem. Senses 33: 709–723, 2008

doi:10.1093/chemse/bjn039
Advance Access publication July 16, 2008

Greater Superficial Petrosal Nerve Transection in Rats does not Change Unconditioned Licking Responses to Putatively Sweet Taste Stimuli

Enshe Jiang[1,2], Ginger Blonde[1,2], Mircea Garcea[2] and Alan C. Spector[1,2]

[1]Department of Psychology and Program in Neuroscience, Florida State University, Tallahassee, FL 32306, USA and [2]Department of Psychology and Center for Smell and Taste, University of Florida, Gainesville, FL 32611, USA

Correspondence to be sent to: Alan C. Spector, Department of Psychology, Florida State University, Tallahassee, FL 32306-4301, USA. e-mail: spector@psy.fsu.edu

Abstract

The greater superficial petrosal nerve (GSP), innervating taste buds in the palate, is known to be exceptionally responsive to sucrose, especially compared with the responsiveness of the chorda tympani nerve (CT). However, whereas transection of the CT (CTX) alone has little or no effect on unconditioned licking responses to many "sweet" stimuli, the impact of GSP transection (GSPX) alone is equivocal. To further examine the role of the GSP on licking responses to putatively sweet-tasting substances, brief-access taste tests were conducted in nondeprived rats before and after sham surgery (SHAM) or CTX or GSPX. A range of concentrations of sucrose, L-alanine, glycine, and L-serine, with and without 1.0 mM inosine monophosphate (IMP) added, were used. All groups showed significant concentration-dependent increases in licking to all stimuli presurgically and postsurgically. CTX decreased licking responses relative to SHAM rats in the first sucrose test. There was also a group × concentration interaction for L-alanine, but post hoc tests did not reveal its basis. Other than this, there were no significant differences among the surgical groups. Interestingly, rats with GSPX tended to initiate fewer trials than SHAM rats. Overall, after GSPX, the remaining gustatory nerves are apparently sufficient to maintain concentration-dependent licking responses to all stimuli tested here. The disparity between our results and others in the literature where GSPX reduced licking responses to sucrose is possibly related to differences in surgical technique or test trial duration.

Key words: amino acids, chorda tympani nerve, deafferentation, gustatory nerves, 5′-inosine monophosphate, sweet taste, umami

Introduction

The greater superficial petrosal nerve (GSP), a branch of the seventh cranial nerve, innervates taste buds in the palate and, in rodents, is exceptionally responsive to sugars (e.g., Nejad 1986; Harada et al. 1997). In at least rats, the most sugar-responsive receptor field innervated by the GSP is found at the opening of the nasoincisor ducts (NID) (Travers et al. 1986; Travers and Norgren 1991). Selective destruction of these receptors, however, has no effect on licking responses to sucrose in a brief-access test across a broad range of concentrations (Spector et al. 1993). Conversely, others have found that GSP transection (GSPX) can severely disrupt licking responses to sucrose (Krimm et al. 1987). The difference in results between these 2 studies could have been due to the fact that in the former study, only the NID was damaged, whereas in the latter, input from the entire GSP was removed. Another possibility is that in the study of Krimm et al. (1987), the transection of the GSP through the middle ear potentially damaged the chorda tympani nerve (CT), which innervates taste buds in the anterior tongue. Transection of the CT has only marginal effects on behavioral responsiveness to sucrose in rats, but combined transection with the GSP causes a more robust attenuation (Krimm et al. 1987; Vigorito et al. 1987; Spector et al. 1993, 1996). Therefore, the role that GSP plays in supporting unconditioned licking responses to sucrose remains to be resolved and represents one focus of this report.

Additionally, little research has been done to determine what nerve input is necessary to support licking responses to amino acids. Several D- and L-amino acids have been shown to possess a taste quality similar to sucrose in various species, based on intake tests and generalization to sucrose in conditioned taste aversion studies, and have been labeled "sweet" by humans (e.g., Schiffman et al. 1981; Ninomiya et al. 1984; Kasahara et al. 1987; Yamamoto et al. 1988;

Danilova et al. 1998; Bachmanov et al. 2001; Manita et al. 2006; Delay et al. 2007; Dotson and Spector 2007; Bachmanov and Beauchamp 2008). It has been shown that most L-amino acids bind to the T1R1 + T1R3 heterodimer, a subset of the T1R family of receptors. However, with the exception of glycine (which is achiral), the L-amino acids do not appear to activate the T1R2 + T1R3 receptor which binds with sugars, artificial sweeteners, and some sweet-tasting D-amino acids (Li et al. 2002; Nelson et al. 2002). Also, in at least rats and mice, the taste buds of the anterior tongue and palate prominently express T1R1, which is only weakly expressed in the posterior tongue, whereas T1R3 is moderately expressed in all 3 taste receptor fields (Montmayeur and Matsunami 2002; Gilbertson and Boughter 2003). These patterns of expression suggest that the CT and GSP might relay important gustatory information in the generation of licking responses to L-amino acids in general, including those thought to have a sucrose-like component. Electrophysiological studies using amino acids as taste stimuli show that both the CT and the GSP are apparently responsive to those compounds (e.g., Harada and Kasahara 2000; Sako et al. 2000; Sakurai et al. 2000).

To help clarify the effects of selective removal of CT or GSP input on behavioral responsiveness to sucrose and some putatively sweet-tasting amino acids, we conducted a series of brief-access licking tests in nondeprived rats using a range of concentrations of sucrose, L-serine, L-alanine, and glycine before and after SHAM surgery or CTX or GSPX. Interestingly, Dotson and Spector (2004) found that in 4 strains of mice, the putatively sweet-tasting amino acids glycine and L-serine did not generate much concentration-dependent licking in a brief-access test when the animals were tested while nondeprived, but these strains still displayed vigorous licking to sucrose. Thus, we wanted to test the generality of this finding in another rodent species such as the rat. Additionally, because neural responsiveness to L-amino acids is enhanced by the addition of 5′-purine nucleotides in a variety of in vivo and in vitro assays (e.g., Sato et al. 1970; Yamashita et al. 1973; Kumazawa et al. 1991; Yamamoto et al. 1991; Sako et al. 2000; Li et al. 2002; Nelson et al. 2002; Maruyama et al. 2006), we tested the behavioral responses of the animals to the stimuli with and without inosine monophosphate (IMP) added. In this regard, Delay et al. (2000) has shown that the lick rates to monosodium glutamate during 30-s trials in nondeprived rats were synergistically increased by the addition of IMP. Most importantly, we used a surgical approach, modified from electrophysiological studies (see Sollars and Hill 2000), to gain access to the GSP through the ventral bulla in an effort to minimize the possibility of inadvertent damage to the CT and thus provide some clarity to the existing disparities in the literature regarding the necessity of GSP input to support unconditioned licking to sucrose in rats—one of the most common animal models in taste research.

Materials and methods

Subjects

Thirty-seven male Sprague–Dawley rats (Charles River Breeders, Wilmington, MA) weighing between 237 and 273 g at arrival served as subjects. The rats were housed individually in separate polycarbonate cages in a colony room except during recovery from surgery, when they were housed in stainless steel hanging wire cages. The rats were maintained in a room in which the lights (12 h light:dark cycle) and temperature were automatically controlled. The rats had access to pelleted chow (LabDiet 5001; PMI Nutrition International Inc., Brentwood, MO) and purified water (filtered reverse osmosis [RO] water; Millipore Elix 10, Bellerica, MA) in their home cage except where noted otherwise. For 4 days before training and testing started, the rats also received ad lib access to an oil mash diet (5 parts powdered chow:2 parts vegetable oil), as they would receive after surgery. All the manipulations were performed during the light phase. All procedures were approved by the Institutional Animal Care and Use Committee at the University of Florida.

Stimuli

All solutions were prepared daily with purified water (filtered RO water; Millipore Elix 10) and reagent grade chemicals and were presented at room temperature. Test stimuli consisted of the 6 different concentrations of sucrose (Fisher Scientific, Atlanta, GA), L-alanine, glycine, L-serine (Sigma-Aldrich, St Louis, MO), and purified water with and without IMP (Sigma-Aldrich) contained in separate tubes. The concentration series for each stimulus was 0.01, 0.03, 0.06, 0.1, 0.3, and 1.0 M.

Stimuli used during electrophysiological recording consisted of 0.5 M NH_4Cl, 0.5 M KCl, 0.1 M citric acid, 1.0 M sucrose (Fisher Scientific), 0.03 M quinine hydrochloride (Sigma-Aldrich), NaCl (0.03, 0.1, 0.3, and 1.0 M) (Fisher Scientific), and NaCl mixed in 100 μM amiloride hydrochloride (Sigma-Aldrich).

Apparatus

A Davis Rig (Davis MS-160, DiLog Instruments, Tallahassee, FL; see Smith 2001; Glendinning et al. 2002) was used to train and test rats. The rats were placed in a plastic rectangular cage (30 × 14.5 × 18 cm) with a wire mesh floor and on specific occasions had access to a single tube from a series of tubes horizontally aligned on a movable mounting block. A motorized shutter covering an oval-shaped opening in the front wall of the chamber controlled access to a slot in a stainless steel panel on the mounting block behind which the orifice of the drinking spout (2.7 mm ± 0.8% diameter) could be reached. Depending on the phase of training and testing, the block could be moved between trials and a new drinking spout positioned behind the shutter.

Behavioral training and testing

Thirty-two animals were used in the behavioral experiment. The procedure is outlined in Table 1. During the training phase, animals were tested under water restriction conditions. They received their daily fluid allotment during the behavioral sessions. Water bottles were removed from the home cage the afternoon before the first day of training. While water restricted, any animal that had a body weight drop of more than 15% of their free-feeding value or that took fewer than 1000 licks during the training session, was given 5 ml supplemental water. On the first day of training, the rats were placed in the Davis Rig and had access to a single bottle of water for 5-s trials to familiarize the animal with the shutter operation and 5-s trial structure. The shutter opened in preparation for a trial, which started as soon as the spout was licked. After 5 s, once the trial was initiated, the shutter was closed for 7.5 s (interpresentation interval) and then reopened for a new trial. The session duration was 40 min for this and all remaining testing and training days. On the second day of training, the rats had access to water through a single stationary tube with the shutter left open for the entire 40-min period during which the animals could lick freely. For the remaining 3 days of training, the animals were again presented with 5-s trials with 7.5-s interpresentation intervals as described above in this section for the first day of training, except with 7 bottles that rotated during the interpresentation interval to emulate the test conditions. For one day, these 7 bottles were filled with water, and for the last 2 training days, the animals received the 6 concentrations of sucrose and water delivered in randomized blocks of 7 under the water restriction conditions. The home cage water bottles were returned after the training session. After a day during which the animals were allowed to rehydrate, testing began under nondeprived conditions.

The animals were tested with concentration series of a single compound in addition to water for 3 consecutive days and then the next compound was tested and so on. On days that a stimulus was mixed with IMP, both water alone and 1 mM IMP in water were also included in the stimulus array. The order of stimulus tests was as follows: sucrose, sucrose + 1 mM IMP, L-alanine, L-alanine + 1 mM IMP, glycine, glycine + 1 mM IMP, L-serine, L-serine + 1 mM IMP, sucrose, sucrose + 1 mM IMP. After testing was completed, the animals received their prescribed surgical treatment, and after 25–35 days of recovery, they were tested again exactly as described above. At the end of behavioral testing, rats in the GSPX and SHAM groups were used for CT electrophysiological recording (see electrophysiology below).

Table 1 Presurgical and postsurgical training and testing protocol

Phase	# of sessions	# of tubes	Water restriction	Concentration varied
Spout training				
Single spout w/5-s trials	1	1	Yes	Water
Stationary spout w/40-min access	1	1	Yes	Water
Multiple 5-s trials	1	7	Yes	Water
Sucrose training	2	7	Yes	Yes
Testing				
Stimulus	3 each	7	No	Yes
Stimulus + IMP	3 each	8	No	Yes

Surgery

Of the 32 rats used in the behavioral experiment, 10 animals were removed from the experiment after presurgical testing because they took less than 2 trials to one or more of the concentrations (including water) of a given stimulus during presurgical testing. The remaining 22 animals were assigned to have 1 of 3 surgeries as described below in this section. An effort was made to balance the surgical groups on the basis of performance during sucrose test sessions, body mass, and testing apparatus.

The rats were deeply anesthetized with an intramuscular injection of a mixture of ketamine hydrochloride (125 mg/kg body mass) and xylazine hydrochloride (5 mg/kg body mass). Supplemental doses were administered as necessary. Additionally, each rat was administered penicillin G Procaine (~30 000 units subcutaneous) and ketorolac tromethamine (2 mg/kg body weight subcutaneous) on the day of surgery. For the 7 rats receiving bilateral CTX, the nerve was transected after widening the ear canal and removing the tympanic membrane. The nerve was cauterized along with the tissue at the junction between the tympanic membrane and the auditory canal. This latter manipulation stimulates the production of cerumin, which fills the bulla and helps prevent regeneration. For the 8 rats receiving bilateral GSPX, the ventral external wall of the bulla was exposed underneath the posterior belly of the digastric muscle by retracting the surrounding musculature. A small hole was placed in the bulla, the tensor tympani was carefully avulsed, and the GSP was exposed under the temporal bone, transected with microscissors, and the ends cauterized. This ventral approach was based on that used by Sollars and Hill (Sollars and Hill 2000; see also Hendricks et al. 2002) and was chosen in an effort to minimize damage to the CT. The 7 rats that received sham surgery (SHAM) had the tympanic membrane punctured and external wall of ventral bulla exposed. Each animal received a subcutaneous injection of penicillin (~30 000 units sc) and ketorolac tromethamine (2 mg/kg body mass sc) on each of 3 days after surgery. In addition, both wet mash (dry powder chow with purified water) and an oil mash diet (as described above) were given daily during the recovery period. Starting on the third day after surgery, a calorically dense nutritional supplement (Nutrical) was mixed into the wet mash. These supplemental

diets were provided to each rat until its body mass increased and stabilized at or above 90% of its presurgical value.

Five additional naive rats, which were not included in the behavioral experiment, received GSPX bilaterally as described above (GSPX-no behavior). During recovery from surgery, these rats also received the supplemental diets as described. This group was used for electrophysiological examination of CT function during the first week of behavioral testing of the other animals to help determine whether the surgical approach to transect the GSP inadvertently damaged the CT and caused any noticeable impairment.

Electrophysiology

Because the approach to the GSP is through the tympanic bulla through which the CT traverses, it is possible that the function of the CT nerve could be inadvertently compromised. We, therefore, electrophysiologically examined CT function in rats at various postsurgical times in a subset of either GSPX or SHAM rats (Table 2) during and after behavioral testing. The GSPX-no behavior group (36–42 days after surgery) served as a control for CT function at the start of behavioral testing for the other GSPX animals. Rats from the SHAM and GSPX groups used in behavioral testing were subdivided to determine whether the CT was compromised after longer times from surgery. The GSPX-early group (78–85 days after surgery) and its accompanying SHAM-early (78–87 days after surgery) controls provided assessment of the CT in a subset of animals immediately after behavioral testing was completed. The GSPX-late (210–214 days after surgery) group and its accompanying SHAM-late (210–211 days after surgery) controls tested whether there were any late developing impairments in CT function over 200 days after GSPX.

The rat was initially anesthetized with an intraperitoneal injection of sodium pentobarbital (75 mg/kg body mass). The level of anesthesia was checked by frequently pinching the hind paw, and supplemental doses were given as needed. Body temperature was monitored and regulated at 36–37 °C by an electric warming pad. After securing the rat in a non-traumatic head holder, tracheal cannulation was performed to facilitate breathing and the hypoglossal nerve was transected bilaterally to prevent movements of the tongue. After exposing the left CT through the mandibular approach, the nerve was dissected free from the surrounding tissue, cut near its entrance to the tympanic bulla, desheathed with 2 microforceps, and hooked with a platinum/iridium electrode. The indifferent electrode was placed in the underlying muscle tissue. Whole nerve neural activity was band-pass filtered (0.3–10 kHz), differentially amplified (Differential AC Amplifier, Model 1700, A-M Systems Inc., Carlsborg, WA), and recorded on a computer using Cambridge Electronic Design hardware and Spike-2 software. Taste stimuli were presented to the anterior tongue via a custom-designed fluid delivery system (DiLog Instruments) at a rate of 0.3 ml/s for 20 s. A 30-s water prerinse and a 40-s water postrinse with purified water (filtered RO water; Millipore Elix-10) preceded and followed the taste stimulus delivery. The flow rate and the time were controlled by a customized program written using the Spike-2 software. There was at least a 95-s rest between 2 stimuli. The order of stimulus presentation was 0.5 M NH_4Cl, 0.5 M KCl, 0.1 M citric acid, 1.0 M sucrose,

Table 2 Groups for postsurgical testing and electrophysiology

Surgical group[a]	Postsurgical testing		Behavioral analysis[b]	Electrophysiology			
	N (surgery and postsurgical testing)	Days from surgery to the start of testing	*N* (behavioral analysis)	Electrophysiological group[d]	*N* (electrophysiological recording)	Days from surgery to electrophysiology	*N* (electrophysiological analysis)[c]
SHAM	7	25–26 days	6	SHAM-early	4	78–87	4
				SHAM-late	3	210–211	3
GSPX	8	33–35 days	5	GSPX-early	4	78–85	3
				GSPX-late	4	210–214	3
				GSPX-no behavior	5	36–42	4
CTX	7	27–28 days	5		N/A[e]	N/A	N/A

[a]Twenty-two rats received surgery and underwent postsurgical testing. An additional 5 rats received GSPX surgery and were used for electrophysiology only (GSPX-no behavior).
[b]Three rats were removed from the behavioral analyses due to histology. One rat was removed due to an abnormally low number of licks to water on 5-s trials during water restriction testing. Two rats were removed from the licking response analyses due to insufficient trials.
[c]Two rats were removed from the electrophysiological analyses due to histology. One rat in the GSPX-no behavior group died during electrophysiological testing and was not included in the analyses. Rats removed from the behavioral analyses for behavioral reasons were included in the electrophysiology.
[d]After postsurgical testing, rats in the SHAM and GSPX groups were further divided into 2 groups for electrophysiological testing: early and late, which correspond to the number of days after surgery when electrophysiological testing occurred.
[e]N/A = not applicable

0.03 M quinine hydrochloride, 0.5 M NH_4Cl, a concentration series of NaCl (0.03, 0.1, 0.3, and 1.0 M), 0.5 M NH_4Cl, a concentration series of NaCl (0.03, 0.1, 0.3 and 1.0 M) mixed in 100 μM amiloride hydrochloride, and 0.5 M NH_4Cl. During NaCl with amiloride presentations, 100 μM amiloride hydrochloride was used as the prerinse and postrinse solution. Here, the 0.5 M NH_4Cl was applied periodically to assess the stability of the preparation, as is common in the literature, because it elicits robust and reliable responses from the rat CT.

Histology

All rats were deeply anesthetized with an intraperitoneal injection of sodium pentobarbital (≥50 mg/kg body mass) and were transcardially perfused with saline followed by 10% buffered formalin. For rats in the CTX group, this occurred after the behavioral testing, and for the rats in the SHAM and GSPX groups, this occurred immediately after electrophysiological recording sessions. The tongue, soft palate, and NID of the rat were removed and stored in 10% buffered formalin. The anterior tongue was cut into 2 halves along the midline after staining with 0.5% methylene blue. The epithelium of each half was pressed between 2 glass slides and observed under a light microscope. The total number of the fungiform papillae and taste pores were subsequently counted. The tissues of the soft palate and the incisive papilla at the opening of the NID were embedded in paraffin and cut into 10-μm sections. The sections were mounted on glass slides and stained with hematoxylin and eosin. Because GSPX causes marked decreases in the number of taste buds in the incisive papilla of the NID and they are easy to count with some accuracy, we used this tissue as a marker for successful GSPX. The one exception was for rat #7 (GSPX), for which the NID tissue was damaged during histological preparation, and therefore, the soft palate was examined. If numerous taste buds were present in the incisive papilla of the NID, the animal was classified as having an intact (or regenerated) GSP and the taste buds were not counted. All tissues were coded so that the counters were blind to the surgical condition.

Data analysis

Only rats that took more than 2 trials to each concentration tested and that had histologically verified transections were included in the behavioral analyses. According to these criteria, 2 rats were removed for their poor performance on trials postsurgically, but we included these 2 rats for the trial number analysis. One additional animal in the SHAM group was discarded because of an abnormally low number of licks to water during postsurgical training. Three rats were discarded from all data analyses because of histological evidence of nerve regeneration or incomplete transections.

The final sample sizes for behavioral analysis were as follows: SHAM, $n = 6$; CTX, $n = 5$; and GSPX, $n = 5$.

The licks per trial taken to each concentration of each stimulus were averaged over the 3-day test period. These values were then standardized by subtracting an individual rat's average licks to water during the test sessions for a given taste stimulus. In this way, the concentration–response curves were constructed relative to a water baseline. When IMP was used as the solvent, the average licks to IMP alone were subtracted from the taste stimulus responses. This measure has been effectively used to assess the effects of nerve transection on sucrose licking in a brief-access test with rats (Spector et al. 1993, 1996).

The distances between the spout orifice and the edge of the slot opening in the lick block were reset at the start of postsurgical testing, and thus, we felt it was prudent to constrain our analysis to a between-subjects examination of presurgical and postsurgical performance. The lick scores were analyzed with a 2-way group (between subjects) × concentration (within subjects) analysis of variance (ANOVA) for each stimulus presurgically or postsurgically. A 1-way ANOVA followed by Bonferroni post hoc tests was applied when there was a significant main effect of group or a group × concentration interaction. The total trials per session taken to each stimulus were averaged over the 3-day test period and analyzed for difference across groups in 1-way ANOVAs followed by Bonferroni post hoc tests when appropriate. To determine the effect of IMP on licking, we conducted separate 2-way ANOVAs (group × IMP) for each stimulus to compare licks to water with licks to IMP alone. To determine whether IMP increased licking to the taste stimuli, we conducted 3-way ANOVAs (group × concentration × IMP). In this latter analysis, we were simply looking for significant main effects of IMP or interaction effects involving IMP.

Rats discarded from the behavioral analyses for histological reasons were also discarded from the electrophysiological analyses. Additionally, one rat in the GSPX-no behavior group died during the electrophysiological recording; data from that animal were not included in the analyses. For the statistical analysis of the electrophysiological data, we first measured the area under the curve (AUC) of the rectified and integrated response (time constant = 0.3 s) for the first 10 s of stimulus application and then divided this value by the average AUC of the rectified and integrated responses to the most recent applications of 0.5 M NH_4Cl before and after the stimulus. In all cases, the AUC of the last 10 s of the water prerinse was first subtracted from the stimulus AUC in the above calculations. A series of 1-way ANOVAs was used to compare GSPX with SHAM rats for responses to KCl, citric acid, quinine, and sucrose. A 3-way group (between subjects) × concentration (within subjects) × amiloride treatment (within subjects) ANOVA was used to analyze CT responses to NaCl. The rejection criterion (e.g., alpha) for all statistical tests was set at the conventional value of 0.05.

Results

Histology

The histological results for different groups are summarized in Table 3. Three rats were excluded from all analyses due to the likelihood of either incomplete transection or nerve regeneration in these rats (see St John et al. 1995, 2003). One rat had 30 taste buds in the soft palate unilaterally and another rat in the GSPX group had 61 taste buds in the NID and so were removed from analyses. A third rat in the CTX group had 29.4% of fungiform papillae with a discernable pore, and although this is borderline, we decided to be prudent and exclude this animal from further analysis.

Licking responses

There were no general impairments in licking behavior after surgery. The mean licks to water during 5-s trials when the animals were tested during water restriction (on the third day) did not significantly differ between the groups before [$F(2,13) = 0.408$, $P = 0.673$] or after [$F(2,13) = 2.019$, $P = 0.172$] surgery (Figure 1). Moreover, there was neither a main effect of group [before surgery: $F(2,13) = 0.594$, $P = 0.567$; after surgery: $F(2,13) = 1.026$, $P = 0.386$] nor a group × concentration interaction [before surgery: $F(12,78) = 0.951$, $P = 0.502$; after surgery: $F(12,78) = 0.770$, $P = 0.679$] in sucrose licking during water restriction (Days 4 and 5) collapsed across trials, but there was a main effect of concentration [before surgery: $F(6,78) = 11.867$, $P < 0.001$; after surgery: $F(6,78) = 18.026$, $P < 0.001$; see Figure 1].

Table 4 provides a summary of the results from 2-way (group × concentration) ANOVAs conducted for each stimulus. The ANOVAs indicated that the concentration effects for each stimulus were all significant (for all stimuli, $P < 0.001$). It is clear from Figures 2– 6 that, both pre- and postsurgically, all groups monotonically increased their licking as the stimulus concentration was raised.

As for group differences in licks relative to water (or IMP) presurgically, there was a main effect of group for the first presurgical test with sucrose. Post hoc analyses indicated that this effect was due to a difference between SHAM and GSPX groups for 0.03 M sucrose (Table 4). Other than this, there were no other group differences presurgically.

Postsurgically, there were few statistically significant effects involving group differences and those that were observed were due to decreases in stimulus licking in the CTX group. There was a main effect of group on licks relative to water for the first sucrose test. Post hoc analysis showed that the effect was due to a significant difference between SHAM and CTX groups for 0.3 M sucrose (Table 4). Although the CTX group licked less to other sucrose concentrations relative to SHAM rats, these differences failed to reach significance. Postsurgically, there was a significant group × concentration interaction measured for L-alanine licks relative to water (see Table 4). However, 1-way ANOVAs of scores conducted for each concentration failed to reveal any group effects.

A series of 3-way ANOVAs (group × IMP × concentration) comparing the adjusted licking scores (tastant licks relative

Table 3 The number of taste buds in anterior tongue and NID across groups

Group	Pores in anterior tongue	Number of intact fungiform papillae	% Intact fungiform papillae containing pore	Number of taste buds in NID
SHAM	156.0 ± 7.82	161.83 ± 8.11	96.4 ± 0.7	Many
GSPX	141.2 ± 8.58	146.2 ± 10.64	97.0 ± 1.7	9.4 ± 3.3
CTX	1.8 ± 0.58	78.8 ± 11.34	2.4 ± 0.8	Many
GSPX-no behavior	139.6 ± 4.31	144.6 ± 4.80	96.6 ± 0.9	4.4 ± 1.5

Values shown are means ± standard errors. The number of the taste buds in the NID was only qualitatively assessed in SHAM and CTX groups.

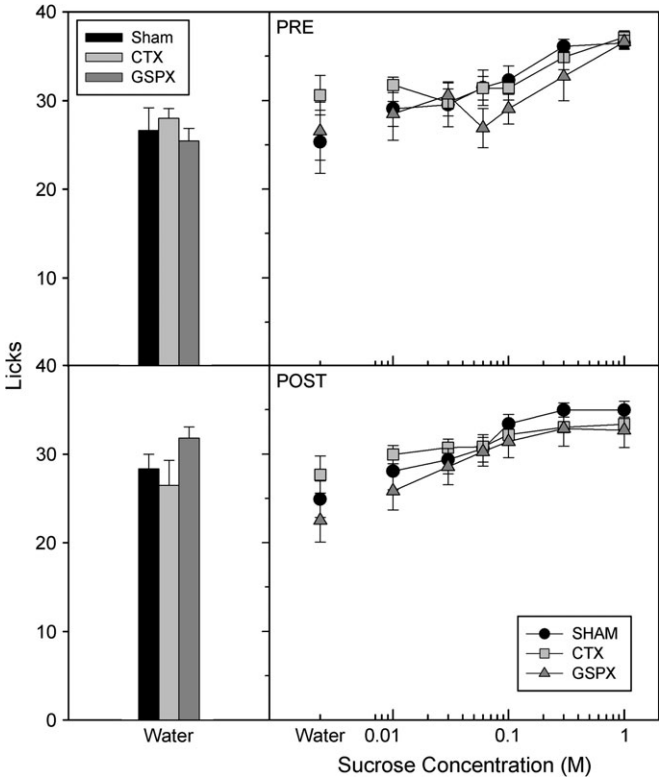

Figure 1 Left: mean (±standard error [SE]) licks to water across groups during 5-s trials on the pre- and postsurgical training when the drinking tubes were moving in between trials and the animals were under a water restriction schedule (i.e., Day 3). Right: mean (±SE) licks to water and 6 concentrations of sucrose across groups during 5-s trials on the pre- and postsurgical training when the drinking tubes were moving in between trials and the animals were under a water restriction schedule (i.e., Days 4 and 5). SHAM, sham-operated control rats (circles); CTX, squares; GSPX, triangles.

Table 4 Summary of ANOVA results for each stimulus across groups

Stimulus	Group	Concentration	Interaction
Sucrose1			
Presurgical	$F(2,13) = 6.176$, $P = \mathbf{0.013}$[a]	$F(6,78) = 125.742$, $P < \mathbf{0.001}$	$F(12,78) = 0.874$, $P = 0.514$
Postsurgical	$F(2,13) = 6.714$, $P = \mathbf{0.012}$[b]	$F(6,78) = 119.68$, $P < \mathbf{0.001}$	$F(12,78) = 1.78$, $P = 0.082$
Sucrose1 + IMP			
Presurgical	$F(2,13) = 0.11$, $P = 0.897$	$F(6,78) = 35.175$, $P < \mathbf{0.001}$	$F(12,78) = 0.629$, $P = 0.784$
Postsurgical	$F(2,13) = 3.426$, $P = 0.064$	$F(6,78) = 70.641$, $P < \mathbf{0.001}$	$F(12,78) = 1.059$, $P = 0.406$
L-alanine			
Presurgical	$F(2,13) = 0.423$, $P = 0.664$	$F(6,78) = 46.848$, $P < \mathbf{0.001}$	$F(12,78) = 0.684$, $P = 0.736$
Postsurgical	$F(2,13) = 1.607$, $P = 0.238$	$F(6,78) = 35.842$, $P < \mathbf{0.001}$	$F(12,78) = 2.16$, $P = \mathbf{0.032}$[c]
L-alanine + IMP			
Presurgical	$F(2,13) = 0.019$, $P = 0.981$	$F(6,78) = 46.407$, $P < \mathbf{0.001}$	$F(12,78) = 1.264$, $P = 0.269$
Postsurgical	$F(2,13) = 1.701$, $P = 0.221$	$F(6,78) = 15.351$, $P < \mathbf{0.001}$	$F(12,78) = 0.953$, $P = 0.492$
Glycine			
Presurgical	$F(2,13) = 1.383$, $P = 0.285$	$F(6,78) = 79.907$, $P < \mathbf{0.001}$	$F(12,78) = 0.502$, $P = 0.883$
Postsurgical	$F(2,13) = 1.64$, $P = 0.232$	$F(6,78) = 81.299$, $P < \mathbf{0.001}$	$F(12,78) = 0.099$, $P = 0.999$
Glycine + IMP			
Presurgical	$F(2,13) = 0.523$, $P = 0.604$	$F(6,78) = 54.955$, $P < \mathbf{0.001}$	$F(12,78) = 1.112$, $P = 0.367$
Postsurgical	$F(2,13) = 2.565$, $P = 0.115$	$F(6,78) = 43.092$, $P < \mathbf{0.001}$	$F(12,78) = 0.948$, $P = 0.497$
L-serine			
Presurgical	$F(2,13) = 0.307$, $P = 0.741$	$F(6,78) = 47.234$, $P < \mathbf{0.001}$	$F(12,78) = 1.084$, $P = 0.388$
Postsurgical	$F(2,13) = 1.505$, $P = 0.258$	$F(6,78) = 26.27$, $P < \mathbf{0.001}$	$F(12,78) = 1.349$, $P = 0.224$
L-serine + IMP			
Presurgical	$F(2,13) = 3.444$, $P = 0.063$	$F(6,78) = 16.55$, $P < \mathbf{0.001}$	$F(12,78) = 0.706$, $P = 0.715$
Postsurgical	$F(2,13) = 1.825$, $P = 0.200$	$F(6,78) = 22.951$, $P < \mathbf{0.001}$	$F(12,78) = 0.474$, $P = 0.901$
Sucrose2			
Presurgical	$F(2,13) = 2.194$, $P = 0.162$	$F(6,78) = 151.088$, $P < \mathbf{0.001}$	$F(12,78) = 0.8$, $P = 0.628$
Postsurgical	$F(2,13) = 1.707$, $P = 0.220$	$F(6,78) = 144.01$, $P < \mathbf{0.001}$	$F(12,78) = 1.324$, $P = 0.237$
Sucrose2 + IMP			
Presurgical	$F(2,13) = 0.123$, $P = 0.850$	$F(6,78) = 86.118$, $P < \mathbf{0.001}$	$F(12,78) = 1.213$, $P = 0.244$
Postsurgical	$F(2,13) = 0.103$, $P = 0.903$	$F(6,78) = 86.116$, $P < \mathbf{0.001}$	$F(12,78) = 1.344$, $P = 0.227$

[a]Post hoc analysis showed that there is a significant difference between the SHAM and GSPX groups for 0.03 M sucrose ($P = 0.027$).
[b]Post hoc analysis showed that there is a significant difference between the SHAM and CTX groups for 0.3 M sucrose ($P = 0.012$).
[c]Post hoc analysis showed no significant group differences at any concentration.
The bold p-values indicate those that were statistically significant.

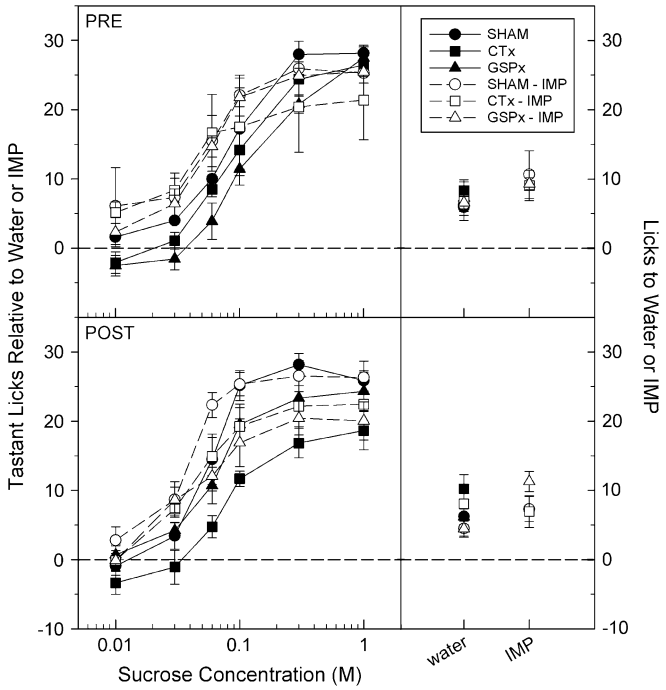

Figure 2 Mean (±standard error) tastant licks relative to water or IMP (stimulus licks minus either water licks during the test sessions or IMP alone licks during test sessions in which IMP was included) as a function of sucrose1 (first phase testing) (solid symbols) and sucrose1 + 1.0 mM IMP (open symbols) concentrations for 3 different groups for presurgical (upper panel) and postsurgical (lower panel) testing. SHAM, sham-operated control rats (circles); CTX, squares; GSPX, triangles.

to water vs. tastant licks relative to IMP) indicated that for all taste stimuli either presurgically or postsurgically, the addition of IMP significantly increased licking, at least at some concentrations (Table 5). For each stimulus, there was always a significant IMP × concentration interaction. This was apparently the case for all groups because there were no interaction terms that included both group and IMP that were significant. Table 6 lists the 2-way ANOVA

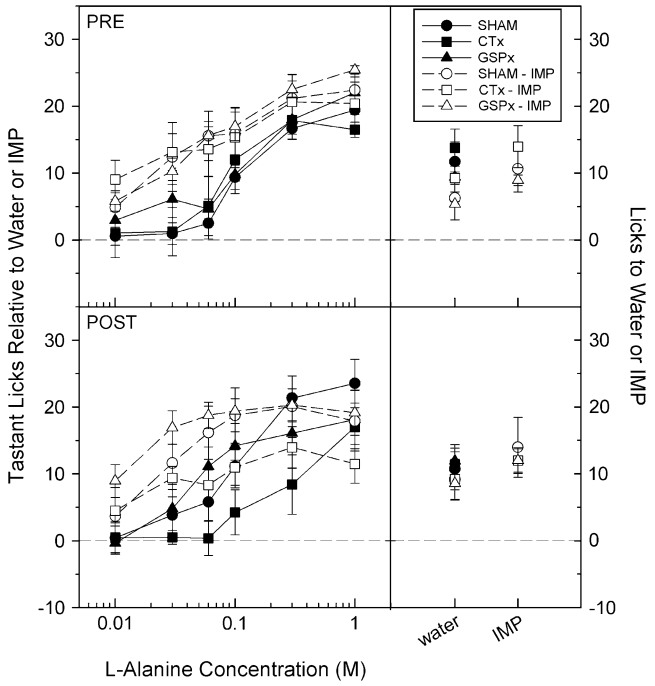

Figure 3 Mean (±standard error) tastant licks relative to water or IMP (stimulus licks minus either water licks during the test sessions or IMP alone licks during test sessions in which IMP was included) as a function of L-alanine (solid symbols) and L-alanine + 1.0 mM IMP (open symbols) concentrations for 3 different groups for presurgical (upper panel) and postsurgical (lower panel) testing. SHAM, sham-operated control rats (circles); CTX, squares; GSPX, triangles.

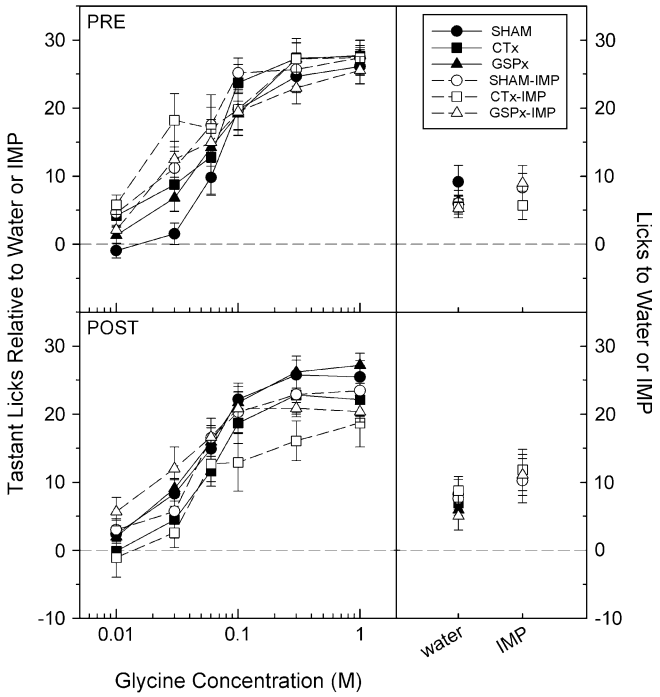

Figure 4 Mean (±standard error) tastant licks relative to water or IMP (stimulus licks minus either water licks during the test sessions or IMP alone licks during test sessions in which IMP was included) as a function of glycine (solid symbols) and glycine + 1.0 mM IMP (open symbols) concentrations for 3 different groups for presurgical (upper panel) and postsurgical (lower panel) testing. SHAM, sham-operated control rats (circles); CTX, squares; GSPX, triangles.

(group × IMP) results for each taste stimulus test pre- and postsurgically in which unadjusted licks to water (on IMP test sessions) were compared with unadjusted licks to IMP alone. Although IMP alone appeared in some cases to increase licking relative to water, the IMP-induced enhancement of licking to taste stimuli cannot be attributed to this because licks to IMP alone were subtracted out from the scores.

Total number of trials

The 2 rats that were excluded from the analysis of licking above because they did not meet the criterion number of trials for inclusion were included in the analysis of trials because their failure to initiate sufficient trials occurred postsurgically and thus could be construed as a potential effect of the surgery. Presurgically, after the animals that did not meet the inclusion criterion were discarded, there were no significant differences in the number of trials taken by the groups for any of the stimuli (see Figure 7, Table 7). However, based on 1-way ANOVAs followed by Bonferroni post hoc tests, the GSPX group did initiate fewer trials than the SHAM group in the early series of tests postsurgically including sucrose, sucrose + IMP, and L-alanine; the L-alanine + IMP result was marginal. The same analysis indicated that the GSPX rats took fewer trials than the SHAM group during sessions with L-serine + IMP. The SHAM and CTX groups did not significantly differ in the number of trials taken.

Electrophysiology

Representative traces of rectified and integrated CT responses to the stimuli applied to the anterior tongue for 2 animals are shown in Figure 8. Figure 9 depicts the mean ratios of the AUC for each taste stimulus relative to the AUC for 0.5 M NH_4Cl for all the groups. A 1-way ANOVA revealed that there were no significant differences across groups for 0.5 M KCl [$F(4,12) = 0.366$, $P = 0.828$], 0.1 M citric acid [$F(4,12) = 2.382$, $P = 0.110$], 1.0 M sucrose [$F(4,12) = 1.058$, $P = 0.419$], and 0.03 M quinine hydrochloride [$F(4,12) = 2.759$, $P = 0.077$]. The CT responded to NaCl in a clearly concentration-dependent manner in all the groups [$F(3,36) = 570.356$, $P < 0.001$]. Moreover, amiloride treatment decreased responsiveness to NaCl by approximately 52.9% [$F(1,12) = 167.74$, $P < 0.001$], and this suppression was similar in all the groups. Therefore, we found no electrophysiological evidence to suggest that our surgical approach to the GSP impaired the function of the CT.

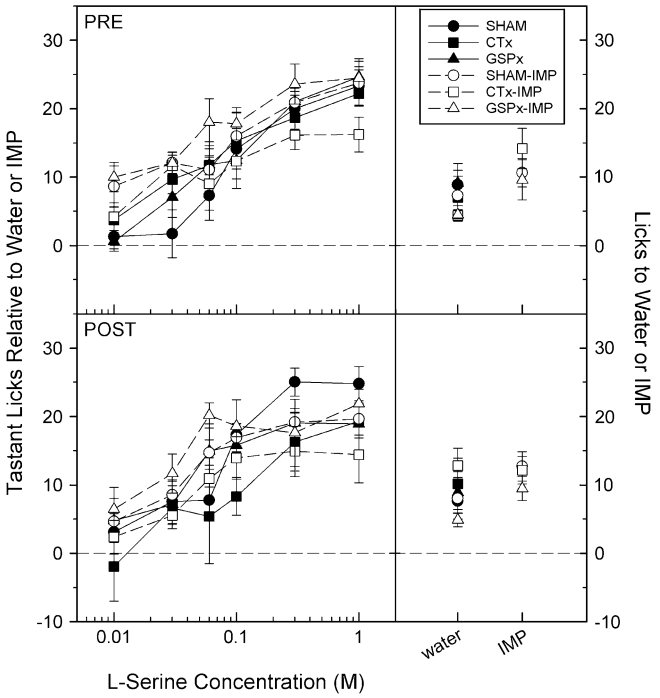

Figure 5 Mean (±standard error) tastant licks relative to water or IMP (stimulus licks minus either water licks during the test sessions or IMP alone licks during test sessions in which IMP was included) as a function of L-serine (solid symbols) and L-serine + 1.0 mM IMP (open symbols) concentrations for 3 different groups for presurgical (upper panel) and postsurgical (lower panel) testing. SHAM, sham-operated control rats (circles); CTX, squares; GSPXtriangles.

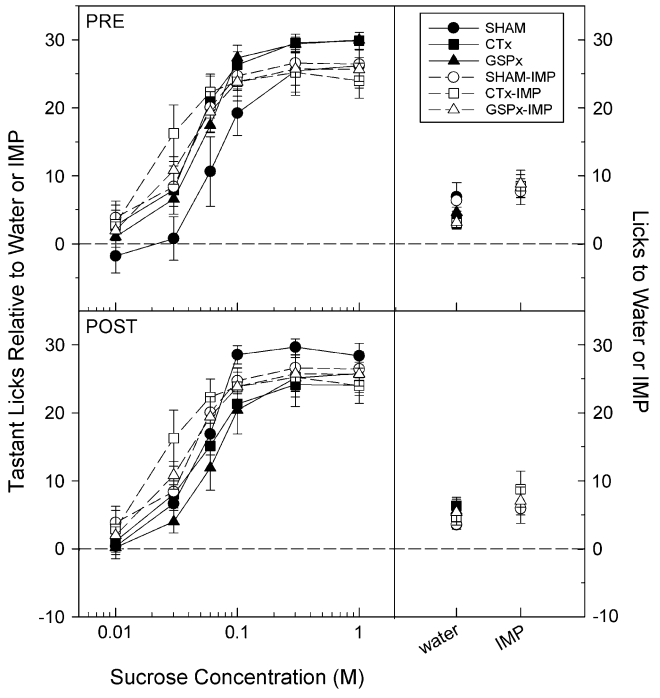

Figure 6 Mean (±standard error) tastant licks relative to water or IMP (stimulus licks minus either water licks during the test sessions or IMP alone licks during test sessions in which IMP was included) as a function of sucrose2 (second phase testing) (solid symbols) and sucrose2 + 1.0 mM IMP (open symbols) concentrations for 3 different groups for presurgical (upper panel) and postsurgical (lower panel) testing. SHAM, sham-operated control rats (circles); CTX, squares; GSPX, triangles.

Discussion

Concentration-dependent licking responses in GSPX rats were no different from those in SHAM rats to any of the stimuli tested here postsurgically. This is consistent with prior work showing that removal of the NID taste receptors (Spector et al. 1993) or transection of the anterior palatine nerve (Yamamoto and Asai 1986; Vigorito et al. 1987) has no effect on sucrose responsiveness in rats. Although this is somewhat surprising because of the exceptional responsiveness of the palatal taste receptors to sucrose, it is clear that the signals from taste buds in the remaining oral fields innervated by the CT and glossopharyngeal nerve (GL) (and perhaps the superior laryngeal nerve) are capable of maintaining the relative affective value of the various sucrose concentrations as well as some putative amino acid sweeteners as assessed in the brief-access taste test.

There was an effect of CTX on licking during the first sucrose test, but only at 0.3 M, and there was an effect on L-alanine licking, although in the latter case the difference failed to reach significance in the Bonferroni post hoc analysis. Overall, these effects, while clearly evident in the curves, were relatively small. This coincides with previous work showing that CTX has marginal effects on unconditioned licking responses to sucrose and maltose in rats (Spector et al. 1993, 1996). Given that combined transection of the gustatory branches of the seventh cranial nerve (i.e., CT and GSP) consistently blunts licking responsiveness to sucrose across a range of concentrations in nondeprived rats (Krimm et al. 1987; Spector et al. 1993, 1996), the result that single nerve transections alone are relatively ineffective suggests that 2 lines of peripheral input arising from the palate and anterior tongue, respectively, converge on central neurons and each is sufficient to maintain responsiveness to sweeteners in this task (for discussion, see Spector et al. 1993). Indeed, there is evidence that some neurons in the central gustatory system respond to taste stimulation of the palate and the anterior tongue (Travers et al. 1986; Travers and Norgren 1991). Alternatively, there might be a threshold number of taste receptor loss before deficits in concentration-dependent licking in response to sweeteners are manifest. These 2 possibilities are not mutually exclusive. Interestingly, GL transection alone in rats is also without effect on licking responses to sucrose as measured in a brief-access test (Spector et al. 1996). Yet, the residual concentration-dependent responding to sucrose by rats after combined

Table 5 Summary of 3-way ANOVA results for each stimulus

Stimulus	Group	IMP	Conc	IMP × group	Conc × group	IMP × conc	IMP × conc × group
Presurgical	$F(2,13) = 0.70$	$F(1,13) = 5.21$	$F(5,65) = 87.32$	$F(2,13) = 0.54$	$F(10,65) = 0.45$	$F(5,65) = 12.7$	$F(10,65) = 0.47$
Sucrose1	$P = 0.516$	$P =$ **0.04**	$P <$ **0.001**	$P = 0.598$	$P = 0.916$	$P <$ **0.001**	$P = 0.474$
Presurgical	$F(2,13) = 0.18$	$F(1,13) = 11.12$	$F(5,65) = 77.28$	$F(2,13) = 0.06$	$F(10,65) = 0.67$	$F(5,65) = 5.10$	$F(10,65) = 1.22$
L-alanine	$P = 0.838$	$P =$ **0.005**	$P <$ **0.001**	$P = 0.94$	$P = 0.745$	$P <$ **0.001**	$P = 0.297$
Presurgical	$F(2,13) = 0.74$	$F(1,13) = 2.71$	$F(5,65) = 93.83$	$F(2,13) = 1.05$	$F(10,65) = 0.81$	$F(5,65) = 0.68$	$F(10,65) = 0.74$
Glycine	$P = 0.496$	$P = 0.124$	$P <$ **0.001**	$P = 0.378$	$P = 0.625$	$P <$ **0.001**	$P = 0.681$
Presurgical	$F(2,13) = 0.75$	$F(1,13) = 3.46$	$F(5,65) = 51.84$	$F(2,13) = 2.87$	$F(10,65) = 1.26$	$F(5,65) = 4.43$	$F(10,65) = 0.40$
L-serine	$P = 0.492$	$P = 0.086$	$P <$ **0.001**	$P = 0.093$	$P = 0.274$	$P =$ **0.008**	$P = 0.943$
Presurgical	$F(2,13) = 1.18$	$F(1,13) = 0.703$	$F(5,65) = 182.9$	$F(2,13) = 1.66$	$F(10,65) = 1.27$	$F(5,65) = 10.3$	$F(10,65) = 0.75$
Sucrose2	$P = 0.339$	$P = 0.417$	$P <$ **0.001**	$P = 0.229$	$P = 0.264$	$P <$ **0.001**	$P = 0.676$
Postsurgical	$F(2,13) = 6.41$	$F(1,13) = 5.35$	$F(5,65) = 119.0$	$F(2,13) = 2.93$	$F(10,65) = 1.46$	$F(5,65) = 7.98$	$F(10,65) = 1.14$
Sucrose1	$P =$ **0.012**	$P =$ **0.038**	$P <$ **0.001**	$P = 0.089$	$P = 0.177$	$P <$ **0.001**	$P = 0.347$
Postsurgical	$F(2,13) = 2.57$	$F(1,13) = 6.58$	$F(5,65) = 35.05$	$F(2,13) = 0.83$	$F(10,65) = 1.88$	$F(5,65) = 11.2$	$F(10,65) = 1.14$
L-alanine	$P = 0.115$	$P =$ **0.023**	$P <$ **0.001**	$P = 0.825$	$P = 0.065$	$P <$ **0.001**	$P = 0.349$
Postsurgical	$F(2,13) = 2.87$	$F(1,13) = 2.67$	$F(5,65) = 73.20$	$F(2,13) = 0.36$	$F(10,65) = 0.28$	$F(5,65) = 7.65$	$F(10,65) = 1.75$
Glycine	$P = 0.093$	$P = 0.126$	$P <$ **0.001**	$P = 0.707$	$P = 0.984$	$P <$ **0.001**	$P = 0.089$
Postsurgical	$F(2,13) = 2.73$	$F(1,13) = 0.522$	$F(5,65) = 40.64$	$F(2,13) = 0.27$	$F(10,65) = 1.18$	$F(5,65) = 3.72$	$F(10,65) = 0.77$
L-serine	$P = 0.102$	$P = 0.483$	$P <$ **0.001**	$P = 0.768$	$P = 0.32$	$P =$ **0.005**	$P = 0.656$
Postsurgical	$F(2,13) = 0.73$	$F(1,13) = 3.01$	$F(5,65) = 141.4$	$F(2,13) = 0.86$	$F(10,65) = 1.51$	$F(5,65) = 7.03$	$F(10,65) = 0.93$
Sucrose2	$P = 0.502$	$P = 0.106$	$P <$ **0.001**	$P = 0.445$	$P = 0.155$	$P <$ **0.001**	$P = 0.515$

Conc, concentration.

CTX and GSPX is abolished by the additional transection of the GL. The fact that the consequences of gustatory nerve transection on unconditioned licking responses to sucrose sum in a nonlinear fashion suggests that the input of all 3 major gustatory nerves contributes in a literally complex fashion to the affective processing of this sugar and likely other sweeteners as well.

We cannot rule out that any effects of CTX on licking to the taste stimuli tested here were due to partial denervation of the sublingual and submandibular salivary glands. However, as noted above, the effects of CTX on taste responsiveness were relatively minor. Moreover, the parasympathetic innervation of the submandibular gland supplied by the lingual nerve proper as well as the sympathetic innervation of both glands provided by the superior cervical ganglion should have remained intact (Hellekant and Kasahara 1973; Young and Van Lennep 1978).

In contrast to the present results, prior work by Krimm et al. (1987) showed that after GSPX, mean licks for 3 low concentrations of sucrose (0.01, 0.03, and 0.1 M) were slightly attenuated, and there was a marked decrease in licking to 0.3 and 1.0 M sucrose in a brief-access test. The difference in the outcomes of the 2 experiments has at least 2 possible origins. First, the disparity might be due to the surgical procedures used. In the experiment of Krimm et al. (1987), the GSP was transected in the middle ear through the auditory meatus in which the CT is vulnerable to inadvertent damage (Spector et al. 1996). Thus, perhaps some of the animals had GSPX combined with CT damage—a condition that uniformly disrupts sucrose responsiveness across studies. In our experiment, we attempted to minimize damage to the CT by using an approach through the ventral bulla (Sollars and Hill 2000). Electrophysiological and histological observations confirmed that the CT is intact and fully functional when the GSP is transected in this fashion.

Alternatively, a second, more intriguing, explanation for the disparity between the 2 studies in the effects of GSPX alone on sucrose licking in rats is rooted in the methodological parameters chosen for the brief-access test. In the present study, after GSPX, animals initiated fewer trials than SHAM rats to some of the stimuli. This was more pronounced for

Table 6 Summary of ANOVA results for IMP alone versus water (water in stimulus with and without IMP testing sessions) across groups

Stimulus	Group	IMP	Interaction
Presurgical			
Sucrose1 + IMP			
Water-IMP	$F(2,13) = 0.022$, $P = 0.978$	$F(1,13) = 3.461$, $P = 0.086$	$F(2,13) = 0.794$, $P = 0.473$
Water (IMP)[a]–IMP	$F(2,13) = 0.019$, $P = 0.981$	$F(1,13) = 5.609$, $P =$ **0.034**	$F(2,13) = 0.287$, $P = 0.755$
Water–water (IMP)	$F(2,13) = 0.420$, $P = 0.666$	$F(1,13) = 0.306$, $P = 0.590$	$F(2,13) = 0.229$, $P = 0.799$
Presurgical			
L-alanine + IMP			
Water–IMP	$F(2,13) = 0.928$, $P = 0.420$	$F(1,13) = 0.057$, $P = 0.816$	$F(2,13) = 0.138$, $P = 0.873$
Water (IMP)–IMP	$F(2,13) = 1.722$, $P = 0.217$	$F(1,13) = 72.747$, $P =$ **0.050**	$F(2,13) = 0.729$, $P = 0.501$
Water–water (IMP)	$F(2,13) = 0.241$, $P = 0.789$	$F(1,13) = 0.264$, $P = 0.616$	$F(2,13) = 0.512$, $P = 0.611$
Presurgical			
Glycine + IMP			
Water–IMP	$F(2,13) = 0.783$, $P = 0.477$	$F(1,13) = 1.441$, $P = 0.251$	$F(2,13) = 0.431$, $P = 0.659$
Water (IMP)–IMP	$F(2,13) = 0.318$, $P = 0.733$	$F(1,13) = 3.112$, $P = 0.101$	$F(2,13) = 3.112$, $P = 0.501$
Water–water (IMP)	$F(2,13) = 0.611$, $P = 0.558$	$F(1,13) = 0.210$, $P = 0.654$	$F(2,13) = 0.932$, $P = 0.418$
Presurgical			
L-serine + IMP			
Water–IMP	$F(2,13) = 0.332$, $P = 0.723$	$F(1,13) = 9.949$, $P =$ **0.008**	$F(2,13) = 0.894$, $P = 0.433$
Water (IMP)–IMP	$F(2,13) = 14.957$, $P = 0.690$	$F(1,13) = 16.616$, $P <$ **0.001**	$F(2,13) = 1.672$, $P = 0.226$
Water–water (IMP)	$F(2,13) = 0.077$, $P = 0.916$	$F(1,13) = 4.956$, $P =$ **0.044**	$F(2,13) = 2.044$, $P = 0.169$
Presurgical			
Sucrose2 + IMP			
Water–IMP	$F(2,13) = 1.282$, $P = 0.310$	$F(2,13) = 2.623$, $P = 0.129$	$F(1,13) = 0.269$, $P = 0.768$
Water (IMP)–IMP	$F(2,13) = 0.176$, $P = 0.840$	$F(1,13) = 17.970$, $P <$ **0.001**	$F(2,13) = 16.764$, $P = 0.150$
Water–water (IMP)	$F(2,13) = 0.175$, $P = 0.842$	$F(1,13) = 14.541$, $P =$ **0.002**	$F(2,13) = 2.285$, $P = 0.141$
Postsurgical			
Sucrose1 + IMP			
Water–IMP	$F(2,13) = 2.495$, $P = 0.121$	$F(1,13) = 7.743$, $P =$ **0.016**	$F(2,13) = 0.039$, $P = 0.962$
Water (IMP)–IMP	$F(2,13) = 0.606$, $P = 0.560$	$F(1,13) = 6.319$, $P =$ **0.026**	$F(2,13) = 3.89$, $P =$ **0.047**
Water–water (IMP)	$F(2,13) = 0.658$, $P = 0.534$	$F(1,13) = 0.741$, $P = 0.405$	$F(2,13) = 4.341$, $P = 0.036$
Postsurgical			
L-alanine + IMP			
Water–IMP	$F(2,13) = 0.006$, $P = 0.994$	$F(1,13) = 4.960$, $P =$ **0.044**	$F(2,13) = 0.242$, $P = 0.789$
Water (IMP)–IMP	$F(2,13) = 0.059$, $P = 0.943$	$F(1,13) = 6.812$, $P =$ **0.022**	$F(2,13) = 0.196$, $P = 0.824$
Water–water (IMP)	$F(2,13) = 0.017$, $P = 0.983$	$F(1,13) = 0.776$, $P = 0.394$	$F(2,13) = 0.471$, $P = 0.635$

Table 6 Continued

Stimulus	Group	IMP	Interaction
Postsurgical			
Glycine + IMP			
Water–IMP	$F(2,13) = 0.770$, $P = 0.483$	$F(1,13) = 0.016$, $P = 0.901$	$F(2,13) = 0.822$, $P = 0.461$
Water (IMP)–IMP	$F(2,13) = 0.269$, $P = 0.768$	$F(1,13) = 10.555$, $P =$ **0.006**	$F(2,13) = 0.788$, $P = 0.475$
Water–water (IMP)	$F(2,13) = 0.044$, $P = 0.958$	$F(1,13) = 16.414$, $P <$ **0.001**	$F(2,13) = 1.054$, $P = 0.376$
Postsurgical			
L-serine + IMP			
Water–IMP	$F(2,13) = 0.835$, $P = 0.456$	$F(1,13) = 0.058$, $P = 0.813$	$F(2,13) = 2.167$, $P = 0.154$
Water (IMP)–IMP	$F(2,13) = 1.865$, $P = 0.194$	$F(1,13) = 4.064$, $P = 0.065$	$F(2,13) = 1.519$, $P = 0.255$
Water–water (IMP)	$F(2,13) = 0.168$, $P = 0.847$	$F(1,13) = 3.113$, $P = 0.101$	$F(2,13) = 0.874$, $P = 0.433$
Postsurgical			
Sucrose2 + IMP			
Water–IMP	$F(2,13) = 1.168$, $P = 0.342$	$F(1,13) = 1.092$, $P = 0.315$	$F(2,13) = 0.870$, $P = 0.442$
Water (IMP)–IMP	$F(2,13) = 0.674$, $P = 0.540$	$F(1,13) = 3.203$, $P = 0.097$	$F(2,13) = 0.215$, $P = 0.810$
Water–water (IMP)	$F(2,13) = 1.003$, $P = 0.393$	$F(1,13) = 2.587$, $P = 0.132$	$F(2,13) = 0.063$, $P = 0.939$

[a]Water (IMP) indicates licks to water alone on days when stimuli were mixed with IMP.

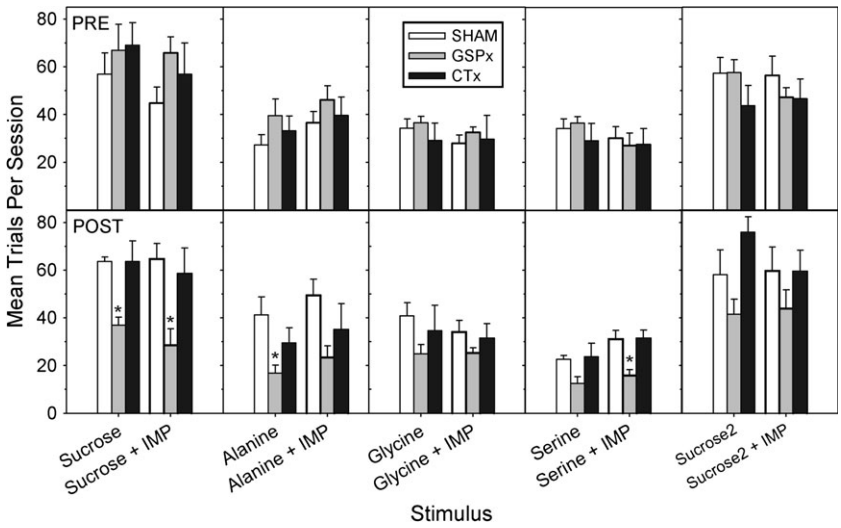

Figure 7 Mean (±standard error) trials taken to each stimulus during 40-min testing sessions. Asterisk represents a significant difference ($P < 0.05$) from the SHAM group. The upper panels show the result from presurgical testing and the lower panels show the result from the postsurgical testing. SHAM, sham-operated control rats.

the first few stimuli tested postsurgically. Indeed, during the initial postsurgical test with sucrose, GSPX animals took almost half as many trials as SHAM rats. This finding suggests that under some circumstances, loss of GSP input can curtail spout approach (i.e., appetitive behavior) while leaving consummatory behavior (licking responses elicited by the stimulus upon contact with the receptors) intact (for more discussion on taste-related appetitive vs. consummatory behavior, see Grill et al. 1987). If true, the fact that the trial duration in the study of Krimm et al. (1987) was 6 times longer (30 s) than that in the present study (5 s) may have allowed the possible effect of GSPX on appetitive initiation of licking bursts to influence the total licks to sucrose measured in the former experiment. This would suggest that the relative contribution of input from various gustatory nerves to appetitive responsiveness can vary from the relative

contribution of that input to consummatory responsiveness. Indeed, a variety of neural manipulations have been shown to dissociate these 2 dimensions of hedonic processing (e.g., Grill and Norgren 1978; Berridge 1996). In the present case, perhaps the central circuits governing appetitive behavior toward sucrose are more affected by the loss of GSP input than those governing consummatory responses.

L-serine, L-alanine, and glycine are putatively sweet tasting to rodents based on the results from conditioned taste aversion generalization tests and intake preference studies (Ninomiya et al. 1984; Kasahara et al. 1987; Yamamoto et al. 1988; Danilova et al. 1998; Delay et al. 2007; Dotson and Spector 2007). Although glycine has been shown, in a heterologous expression system, to activate cells containing the mouse T1R2 + T1R3 heterodimer, the receptor known to be critical in the generation of sucrose taste, L-serine, and L-alanine apparently does not (Nelson et al. 2001). Also, deletion of the T1R2 subunit in mice virtually eliminates sucrose responsiveness but has no effect on L-serine or L-alanine licking in a brief-access test (Zhao et al. 2003). All 3 amino acids have been shown to activate cells containing the T1R1 + 3 heterodimer—a receptor to which, in mice, most common L-amino acids bind and has been promoted as critical in the generation of umami taste (Nelson et al. 2002). Our study was not designed to assess the qualitative perceptual properties of these stimuli, but it is quite clear that all the stimuli were effective at generating licking responses in at least a subset of nondeprived rats. This contrasts somewhat with the findings in nondeprived mice in several "sweet-sensitive" (such as C57BL/6J and SWR/J) and "insensitive" (such as 129P3/J and DBA/2J) strains, in which L-serine does not generate much concentration-dependent licking and glycine responses are rather weak (Dotson and Spector 2004). However, partial food and water restriction is apparently able to amplify responsiveness to at least L-serine in mice (Zhao et al. 2003). So the difference between rats and mice in the licking response to the amino acid stimuli used here probably relates to species differences in the relative affective potency of the stimuli under different physiological states.

Finally, consistent with prior reports in rodents (e.g., Yamamoto et al. 1991; Delay et al. 2000) and in humans (Kawai et al. 2002), the addition of IMP enhanced responsiveness to the amino acid stimuli. It also enhanced responsiveness to water, but this effect was not always observed. Wifall et al. (2007) has shown that IMP is detectable by rats and preferred in 24-h 2-bottle preference test versus water.

Table 7 ANOVA results for trials taken to each stimulus across groups

Stimulus	Presurgery	Postsurgery
Sucrose1	$F(2,15) = 0.05$, $P = 0.951$	$F(2,15) = 6.07$, $P =$ **0.012**[a]
Sucrose1 + IMP	$F(2,15) = 1.44$, $P = 0.268$	$F(2,15) = 5.58$, $P =$ **0.015**[a]
L-alanine	$F(2,15) = 0.93$, $P = 0.416$	$F(2,15) = 4.90$, $P =$ **0.023**[a]
L-alanine + IMP	$F(2,15) = 0.63$, $P = 0.548$	$F(2,15) = 3.64$, $P = 0.051$
Glycine	$F(2,15) = 0.72$, $P = 0.505$	$F(2,15) = 1.66$, $P = 0.223$
Glycine + IMP	$F(2,15) = 0.10$, $P = 0.906$	$F(2,15) = 1.36$, $P = 0.287$
L-serine	$F(2,15) = 2.31$, $P = 0.134$	$F(2,15) = 2.49$, $P = 0.116$
L-serine + IMP	$F(2,15) = 0.40$, $P = 0.678$	$F(2,15) = 5.51$, $P =$ **0.016**[a]
Sucorse2	$F(2,15) = 1.01$, $P = 0.389$	$F(2,15) = 3.11$, $P = 0.074$
Sucrose2 + IMP	$F(2,15) = 0.93$, $P = 0.418$	$F(2,15) = 1.02$, $P = 0.385$

[a]Post hoc analysis showed that the GSPX group significantly differed from the SHAM group for sucrose, sucrose + IMP (first phase testing), L-alanine, and L-serine + IMP ($P < 0.05$).

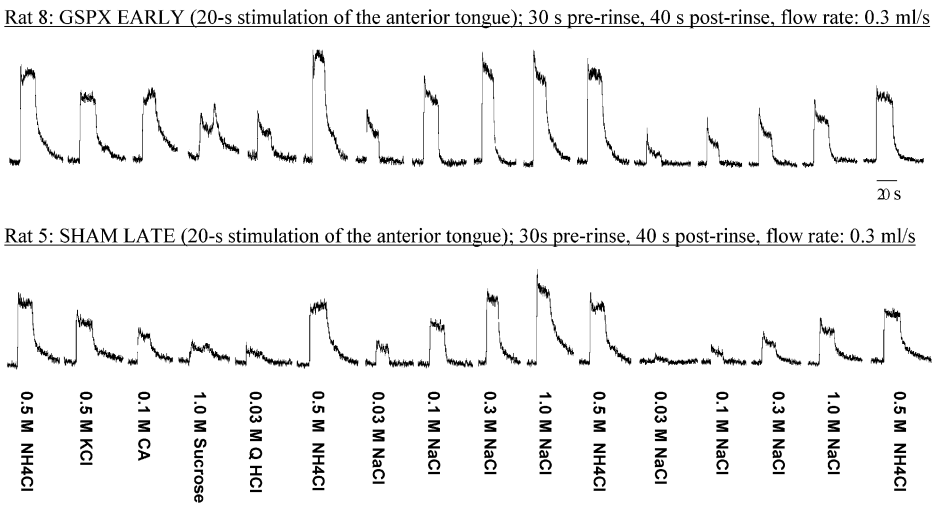

Figure 8 Representative interrupted traces of rectified and integrated CT response from Rat 8 (GSPX-early: CT electrophysiological recording from 84 days after surgery) and Rat 5 (SHAM-late: CT electrophysiological recording from 210 days after surgery). SHAM, sham-operated control rats; CA, citric acid; Q, quinine; A, amiloride hydrochloride.

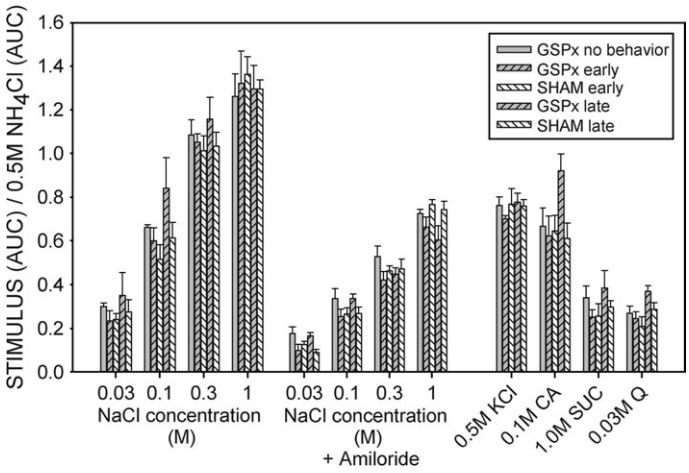

Figure 9 Mean (±standard error) responses of the CT in SHAM rats and rats with GSPX. The bars represent the AUC of the rectified and integrated response (time constant = 0.3 s) for the first 10 s of stimulus application divided by the average rectified and integrated response to the most recent application of 0.5 M ammonium chloride before and after the stimulus. In all cases, the response of the last 10 s of the water prerinse was subtracted out of stimulus AUC in the calculation. CA, citric acid; SUC, sucrose; Q, quinine.

Interestingly, IMP also increased responsiveness to sucrose. In all these cases, however, the increase in responsiveness, although significant, did not appear to be remarkable in magnitude. Moreover, the use of the amino acid stimuli and the addition of the IMP did not substantially reveal any differential effect of the various surgical manipulations on responsiveness in this behavioral assay.

The fact that GSPX, a nerve exceptionally responsive to sucrose in the rat, has no effect on concentration-dependent licking in a brief-access test under the conditions used here highlights the complexity of the functional organization of the peripheral gustatory system. There is not necessarily a direct linear relationship between the overall neural responsiveness of a nerve and the functional consequences of its removal. Indeed, impairments in taste-related tasks following gustatory nerve transection have been shown to depend heavily not only on the targeted nerves but also on the characteristics of the behavioral procedure used to assess function (see Spector 2003). Accordingly, it is quite possible that GSPX might lead to functional deficits involving sucrose or other sweeteners as measured by other taste-related tasks such as those assessing detection thresholds or taste discrimination. In fact, as noted above, when a longer trial duration has been used in the brief-access test, deficits in sucrose licking after GSPX have been revealed. Further studies need to be done to more fully clarify the effect of GSPX on licking responses to sucrose.

Funding

National Institute on Deafness and Other Communication Disorders (R01-DC01628).

Acknowledgements

We would like to thank David Hill for advice on the surgical approach to the GSP, and we would also like to thank Angela Newth, Tiffany von Hartmann, and Nathalie Anderson for their help in all phases of this experiment.

References

Bachmanov AA, Beauchamp GK. 2008. Amino acid and carbohydrate preferences in C57BL/6ByJ and 129P3/J mice. Physiol Behav. 93(1–2): 37–43.

Bachmanov AA, Tordoff MG, Beauchamp GK. 2001. Sweetener preference of C57BL/6ByJ and 129P3/J mice. Chem Senses. 26(7):905–913.

Berridge KC. 1996. Food reward: brain substrates of wanting and liking. Neurosci Biobehav Rev. 20:1–25.

Danilova V, Hellekant G, Tinti JM, Nofre C. 1998. Gustatory responses of the hamster Mesocricetus auratus to various compounds considered sweet by humans. J Neurophysiol. 80:2102–2112.

Delay ER, Beaver AJ, Wagner KA, Stapleton JR, Harbaugh JO, Catron KD, Roper SD. 2000. Taste preference synergy between glutamate receptor agonists and inosine monophosphate in rats. Chem Senses. 25(5): 507–515.

Delay ER, Mitzelfelt JD, Westburg AM, Gross N, Duran BL, Eschle BK. 2007. Comparison of L-monosodium glutamate and L-amino acid taste in rats. Neuroscience. 148(1):266–278.

Dotson CD, Spector AC. 2004. The relative affective potency of glycine, L-serine and sucrose as assessed by a brief-access taste test in inbred strains of mice. Chem Senses. 29(6):489–498.

Dotson CD, Spector AC. 2007. Behavioral discrimination between sucrose and other natural sweeteners in mice: implications for the neural coding of T1R ligands. J Neurosci. 27(42):11242–11253.

Gilbertson TA, Boughter JD. 2003. Taste transduction: appetizing times in gustation. Neuro Rep. 14(7):905–911.

Glendinning JI, Gresack J, Spector AC. 2002. A high-throughput screening procedure for identifying mice with aberrant taste and oromotor function. Chem Senses. 27(5):461–474.

Grill HJ, Norgren R. 1978. The taste reactivity test. I. Mimetic responses to gustatory stimuli in neurologically normal rats. Brain Res. 143:281–297.

Grill HJ, Spector AC, Schwartz GJ, Kaplan JM, Flynn FW. 1987. Evaluating taste effects on ingestive behavior. In: Toates F, Rowland N, editors. Techniques in the behavioral and neural sciences. Vol. 1: feeding and drinking. Amsterdam (The Netherlands): Elsevier. p. 151–188.

Harada S, Kasahara Y. 2000. Inhibitory effect of gurmarin on palatal taste responses to amino acids in the rat. Am J Physiol Regul Integr Comp Physiol. 278(6):R1513–R1517.

Harada S, Yamamoto T, Yamaguchi K, Kasahara Y. 1997. Different characteristics of gustatory responses between the greater superficial petrosal and chorda tympani nerves in the rat. Chem Senses. 22(2): 133–140.

Hellekant G, Kasahara Y. 1973. Secretory fibres in the trigeminal part of the lingual nerve to the mandibular salivary gland of the rat. Acta Physiol Scand. 89:198–207.

Hendricks SJ, Sollars SI, Hill DL. 2002. Injury-induced functional plasticity in the peripheral gustatory system. J Neurosci. 22(19):8607–8613.

Kasahara T, Iwasaki K, Sato M. 1987. Taste effectiveness of some D- and L-amino acids in mice. Physiol Behav. 39:619–624.

Kawai M, Okiyama A, Ueda Y. 2002. Taste enhancements between various amino acids and IMP. Chem Senses. 27(8):739–745.

Krimm RF, Nejad MS, Smith JC, Miller IJ Jr, Beidler LM. 1987. The effect of bilateral sectioning of the chorda tympani and the greater superficial petrosal nerves on the sweet taste in the rat. Physiol Behav. 41: 495–501.

Kumazawa T, Nakamura M, Kurihara K. 1991. Canine taste nerve responses to umami substance. Physiol Behav. 49(5):875–881.

Li X, Staszewski L, Xu H, Durick K, Zoller M, Adler E. 2002. Human receptors for sweet and umami taste. Proc Natl Acad Sci USA. 99:4692–4696.

Manita S, Bachmanov AA, Li X, Beauchamp GK, Inoue M. 2006. Is glycine "sweet" to mice? Mouse strain differences in perception of glycine taste. Chem Senses. 31(9):785–793.

Maruyama Y, Pereira E, Margolskee RF, Chaudhari N, Roper SD. 2006. Umami responses in mouse taste cells indicate more than one receptor. J Neurosci. 26(8):2227–2234.

Montmayeur JP, Matsunami H. 2002. Receptors for bitter and sweet taste. Curr Opin Neurobiol. 12:366–371.

Nejad MS. 1986. The neural activities of the greater superficial petrosal nerve of the rat in response to chemical stimulation of the palate. Chem Senses. 11:283–293.

Nelson G, Chandrashekar J, Hoon MA, Feng L, Zhao G, Ryba NJP, Zuker CS. 2002. An amino-acid taste receptor. Nature. 416(6877):199–202.

Nelson G, Hoon MA, Chandrashekar J, Zhang Y, Ryba NJ, Zuker CS. 2001. Mammalian sweet taste receptors. Cell. 106(3):381–390.

Ninomiya Y, Higashi T, Katsukawa H, Mizukoshi T, Funakoshi M. 1984. Qualitative discrimination of gustatory stimuli in three different strains of mice. Brain Res. 322:83–92.

Sako N, Harada S, Yamamoto T. 2000. Gustatory information of umami substances in three major taste nerves. Physiol Behav. 71(1–2): 193–198.

Sakurai N, Kanemura F, Watanabe K, Shimizu Y, Tonosaki K. 2000. Effects of acids on neural activity elicited by other taste stimuli in the rat chorda tympani. Brain Res. 859(2):369–372.

Sato M, Yamashita S, Ogawa H. 1970. Potentiation of gustatory response to monosodium glutamate in rat chorda tympani fibers by addition of 5′-ribonucleotides. Jpn J Physiol. 20(4):444–464.

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

Smith JC. 2001. The history of the "Davis Rig". Appetite. 36(1):93–98.

Sollars SI, Hill DL. 2000. Lack of functional and morphological susceptibility of the greater superficial petrosal nerve to developmental dietary sodium restriction. Chem Senses. 25(6):719–727.

Spector AC. 2003. The functional organization of the peripheral gustatory system: lessons from behavior. In: Fluharty SJ, Grill HJ, editors. Progress in psychobiology and physiological psychology. Vol. 18. San Diego (CA): Academic Press. p. 101–161.

Spector AC, Redman R, Garcea M. 1996. The consequences of gustatory nerve transection on taste-guided licking of sucrose and maltose in the rat. Behav Neurosci. 110(5):1096–1109.

Spector AC, Travers SP, Norgren R. 1993. Taste receptors on the anterior tongue and nasoincisor ducts of rats contribute synergistically to behavioral responses to sucrose. Behav Neurosci. 107:694–702.

St John SJ, Garcea M, Spector AC. 2003. The time course of taste bud regeneration after glossopharyngeal or greater superficial petrosal nerve transection in rats. Chem Senses. 28(1):33–43.

St John SJ, Markison S, Spector AC. 1995. Salt discriminability is related to number of regenerated taste buds after chorda tympani nerve section in rats. Am J Physiol. 269:R141–R153.

Travers SP, Norgren R. 1991. Coding the sweet taste in the nucleus of the solitary tract: differential roles for anterior tongue and nasoincisor duct gustatory receptors in the rat. J Neurophysiol. 65:1372–1380.

Travers SP, Pfaffmann C, Norgren R. 1986. Convergence of lingual and palatal gustatory neural activity in the nucleus of the solitary tract. Brain Res. 365:305–320.

Vigorito M, Sclafani A, Jacquin MF. 1987. Effects of gustatory deafferentation on polycose and sucrose appetite in the rat. Neurosci Biobehav Rev. 11:201–209.

Wifall TC, Faes TM, Taylor-Burds CC, Mitzelfelt JD, Delay ER. 2007. An analysis of 5′-inosine and 5′-guanosine monophosphate taste in rats. Chem Senses. 32(2):161–172.

Yamamoto T, Asai K. 1986. Effects of gustatory deafferentation on ingestion of taste solutions as seen by licking behavior in rats. Physiol Behav. 37(2):299–305.

Yamamoto T, Matsuo R, Fujimoto Y, Fukunaga I, Miyasaka A, Imoto T. 1991. Electrophysiological and behavioral studies on the taste of umami substances in the rat. Physiol Behav. 49(5):919–925.

Yamamoto T, Matsuo R, Kiyomitsu Y, Kitamura R. 1988. Taste effects of "umami" substances in hamsters as studied by electrophysiological and conditioned taste aversion techniques. Brain Res. 451:147–162.

Yamashita S, Ogawa H, Sato M. 1973. The enhancing action of 5′-ribonucleotide on rat gustatory nerve fiber response to monosodium glutamate. Jpn J Physiol. 23(1):59–68.

Young JA, Van Lennep EW. 1978. The morphology of salivary glands. 1st ed. London: Academic Press.

Zhao GQ, Zhang Y, Hoon MA, Chandrashekar J, Erlenbach I, Ryba NJ, Zuker CS. 2003. The receptors for mammalian sweet and umami taste. Cell. 115(3):255–266.

Accepted June 6, 2008