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Responses of the Rat Chorda Tympani Nerve to Glutamate–Sucrose Mixtures

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Abstract

Monosodium glutamate (MSG) has a multifaceted, unusual taste to humans. Rats and other rodents also detect a complex taste to MSG. Responses of the chorda tympani nerve (CT) to glutamate applied to the front of the tongue were recorded in 13 anesthetized rats. Whole-nerve responses to 30 mM, 100 mM and 300 mM MSG mixed with 300 mM sucrose were recorded before and after adding 30 μM amiloride to the rinse and stimulus solutions. Responses of CT single fibers were also recorded. Predictions from models of whole-nerve responses to binary mixtures were compared to the observed data. Results indicated that MSG-elicited CT responses have multiple sources, even in an amiloride-inhibited environment in rats. Those sources include responses of sucrose-sensitive CT neural units, which may provide the substrate for a sucrose-glutamate perceptual similarity, and responses of sucrose-insensitive CT neural units, which may respond synergistically to MSG–sucrose mixtures.

Key words: mixture models, MSG, single fibers, umami, whole nerve

Introduction

In humans, the taste of monosodium glutamate (MSG) differs from the basic sweet, salty, sour or bitter tastes (Schiffman *et al.*, 1980; Kawamura and Kare, 1987; Hettinger *et al.*, 1996; Halpern, 2002; Ikeda, 2002; Lindemann *et al.*, 2002). Yet, humans and other species also perceive similarities between MSG and basic taste stimuli.

Hamsters and rats perceive similarities between MSG and NaCl, likely by recognizing the taste of the sodium ion. These rodents also perceive similarities between MSG and sucrose when the Na^+-specific taste, thought to be mediated by an epithelial sodium channel (ENaC), is reduced with amiloride (Stapleton *et al.*, 2002). MSG activates chorda tympani (CT) neurons that respond best to sucrose and other neurons that respond to NaCl in hamsters and mice (Yamamoto *et al.*, 1988, 1991; Ninomiya and Funakoshi, 1989a,b; Hettinger and Frank, 1990). Separate candidate molecular taste receptors for sweet and umami taste stimuli have been identified for humans with homologous taste receptors identified for rats and mice (Chaudhari *et al.*, 2000; Li *et al.*, 2002; Nelson *et al.*, 2001, 2002; Damak *et al.*, 2003; Zhao *et al.*, 2003). Thus, MSG may stimulate separate receptors for Na^+, sugars and glutamate (amino acids) in taste bud cells that activate the CT in rats.

The rat CT responds strongly to umami substances, and amiloride, an ENaC blocker, strongly inhibits responses of a subset of rat Na^+-specific CT neurons (Ninomiya and Funakoshi, 1988). The result is a small amiloride-insensitive response to MSG (Yamamoto *et al.*, 1991). Given non-halide sodium salts typically produce little or no CT response after amiloride (Formaker and Hill, 1988), glutamate, rather than sodium, is the likely source of the remaining CT response. Furthermore, when the rat's tongue is treated with amiloride solutions greater than or equal to 30 μM, the specific behavioral identification of Na^+ is lost (Hill *et al.*, 1990; Spector *et al.*, 1996; Sako *et al.*, 2000). Thus, with amiloride present, MSG would primarily activate sugar and glutamate (amino-acid) taste receptors in the rat.

Models have been developed that use responses of the multiple fibers in a taste nerve to binary mixtures to identify the number of sources that independently contribute to a neural response (Hyman and Frank, 1980a,b; Formaker and Frank, 1996). One model, stimulus substitution, assigns mixture responses to a single source; the other model, response additivity, assigns mixture effects to two independent sources. Results that fall outside the range of the

predictions of these two models suggest mixture interactions such as inhibition, if observed responses are lower than stimulus substitution, or synergy, if observed responses are greater than response additivity.

Rat CT whole-nerve responses were recorded to binary mixtures of MSG and sucrose with amiloride added to all solutions and predictions from models of binary mixtures were compared to the observed data. Responses of CT single fibers to MSG, in the presence of amiloride, and sucrose were recorded to determine whether glutamate and sucrose individually activated identical or distinct classes of CT neurons. The studies address the generation of a glutamate taste by the CT of rats (Sako *et al.*, 2000).

Materials and methods

Subjects

Electrophysiological responses were recorded from the right CT nerve of 13 adult male Sprague–Dawley rats: whole-nerve data were collected from eight animals; single-fiber data were collected from five animals. Animals were purchased from Charles River Labs and were individually housed in the vivarium facilities at the University of Connecticut Health Center. The vivarium was maintained at 21°C on a 12 h light–dark cycle (lights on at 07.00). All animals were allowed *ad libitum* access to tap water and Agway Pro-Lab 3000 rodent diet.

Surgical procedure

Rats were initially anesthetized with an intraperitoneal injection of sodium pentobarbital (75 mg/kg) and subsequent injections (30 mg/kg) were given as needed to maintain a surgical level of anesthesia. Body temperature was regulated at 36–37°C with an electronic warming plate or a Deltaphase® isothermal pad (Braintree Scientific). A tracheal cannula was implanted and the hypoglossal nerve was transected bilaterally to prevent tongue movements. With the animal secured in a non-traumatic head holder, the right CT nerve was exposed using a mandibular approach. The CT was cut near its entrance to the tympanic bulla, dissected free of surrounding tissue, desheathed and placed on a nichrome wire recording electrode with an indifferent electrode placed in nearby tissue for whole-nerve recordings. For single-fiber recordings, the CT was further subdivided into fine strands and each strand was placed on the nichrome wire electrode.

Electrophysiology

Multi-fiber neural activity recorded from the whole nerve was differentially amplified, observed with an oscilloscope and audio monitor, squared, filtered (200 ms time-constant), and displayed on a chart recorder. Examples of raw recordings are shown in Figure 1. A transient off response often coincided with the stimulus rinse after solutions containing MSG. Off responses have previously been reported for the rat CT with rinses following other stimuli, such as sucrose (Yamamoto and Kawamura, 1974) and hydrochloric acid (DeSimone *et al.*, 1995).

Whole-nerve response magnitudes were quantified as the average of three response heights measured at 2, 4 and 8 s after stimulus application (Figure 1). This measure of neural response positively correlates ($r = 0.99$, $P < 0.05$) with the total area under an 8 s response curve (measured using ImageTool, a program developed at the University of Texas Health Science Center at San Antonio, available at http://ddsdx.uthscsa.edu/dig/itdesc.html). Thus, this average response measure is highly predictive of a fully integrated measure of whole-nerve activity.

In single-fiber experiments, responses of functional strands were recorded by a Vetter VCR for subsequent offline analyses. Single fiber responses were identified using a PC equipped with RC Electronics' Enhanced Graphics Acquisition and Analyses (EGAA) system. The EGAA system discriminates, counts and graphs single-fiber response trains via its wave shape recognition and histogram analysis modules. Uniform amplitude and waveform shape identified each single fiber for analysis (Figure 2).

Gustatory stimuli

Whole-nerve recordings

Taste stimuli were presented via a gravity flow system at a rate of 2 ml/s for ~10 s followed by a 45 s or longer rinse. The anterior tongue projected through a rubber dam into a glass flow chamber. Compounds were reagent grade, dissolved in distilled water or an aqueous solution of 30 μM amiloride hydrochloride, and presented at room temperature (21°C). Stimuli were presented first without amiloride using water rinses and then again with amiloride using amiloride rinses. When the procedure involved amiloride, 30 μM amiloride was pre-rinsed over the tongue for 2 min and the 30 μM amiloride solution was used as the rinse between stimulus applications. Stimuli consisted of a concentration series (30, 100 and 300 mM) of MSG and sucrose, and the binary combinations of 300 mM sucrose with all concentrations of MSG; 1.0 M sucrose was also used in the sucrose concentration series as it was needed to generate response model predictions for the mixture analysis. Binary-component stimuli were prepared such that the concentration of each component in the mixture was the same as its concentration when presented alone.

Single-fiber recordings

Taste stimuli were presented with a 1 cc syringe modified with a number 23 gauge, 1.5″ blunt needle. Approximately 0.5 ml of each stimulus was applied to the anterior tongue followed ~10–20 s later by two 1 cc distilled water rinses. Stimuli included 300 mM sucrose, 100 mM MSG and 100 mM MSG dissolved in an aqueous solution of 30 μM amiloride.

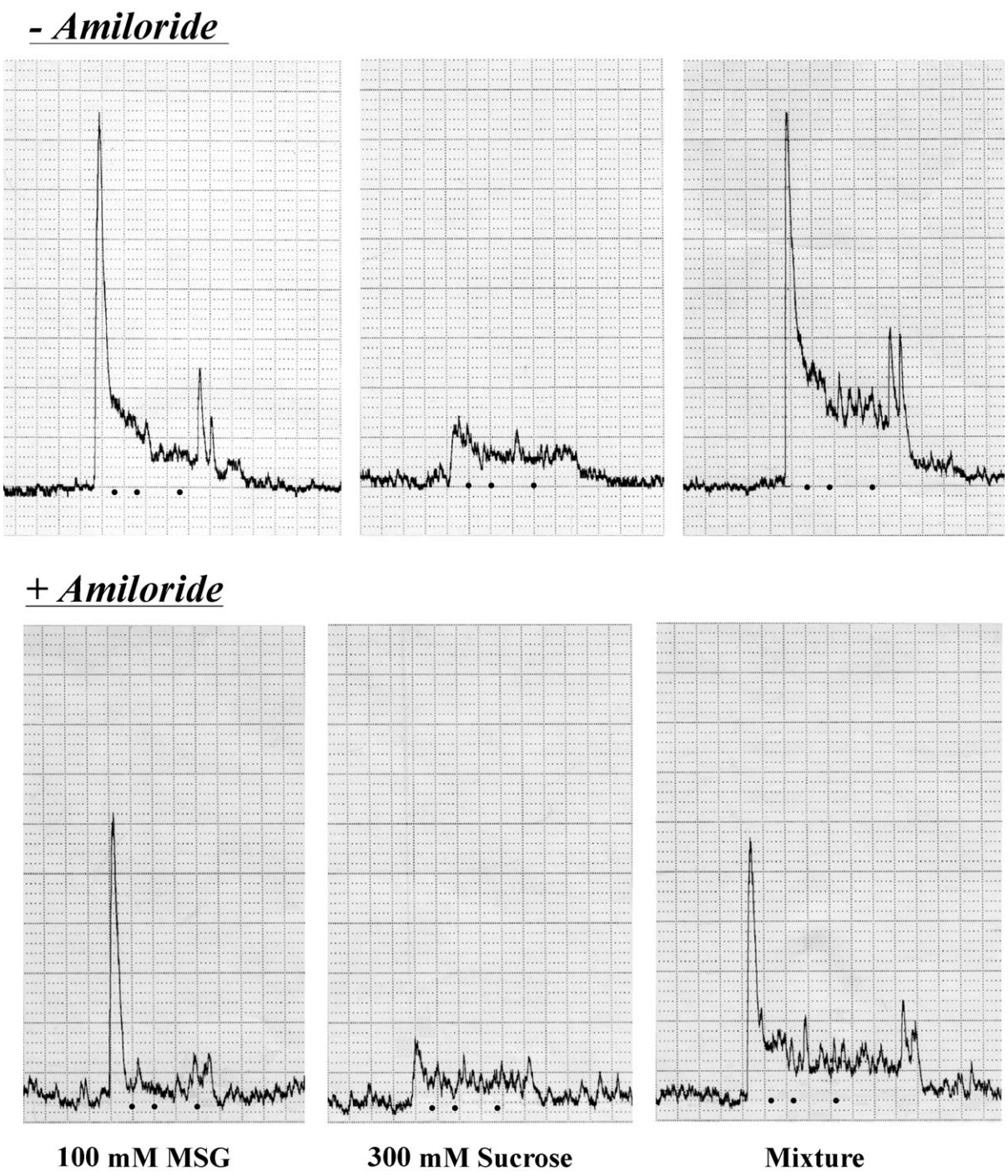

Figure 1 Representative raw data traces for 100 mM MSG, 300 mM sucrose and the mixture of these two stimuli with (+) and without (–) 30 μM amiloride treatment. Stimulus solutions applied with amiloride treatment all contained 30 μM amiloride. The three dots under each trace identify the three time points (2, 4 and 8 s) used to obtain the average neural response. The transient response peak at the beginning of each trace corresponds with stimulus onset. Stimuli flowed over the tongue for ~10 s. The distance between each major vertical division represents 2 s.

Data analyses

Whole-nerve recordings

Only responses from stable recordings were included in the data analysis. The integrity of each preparation was monitored by periodic application of 500 mM NH_4Cl. The NH_4Cl, applied at the beginning and end of each stimulus concentration series, was chosen as the standard stimulus because it elicits reliable responses from the rat CT, it has been used previously as a standard and, of the salts tested, is the least affected by amiloride (Hill and Bour, 1985; Lundy and Contreras, 1997). A recording for a concentration series

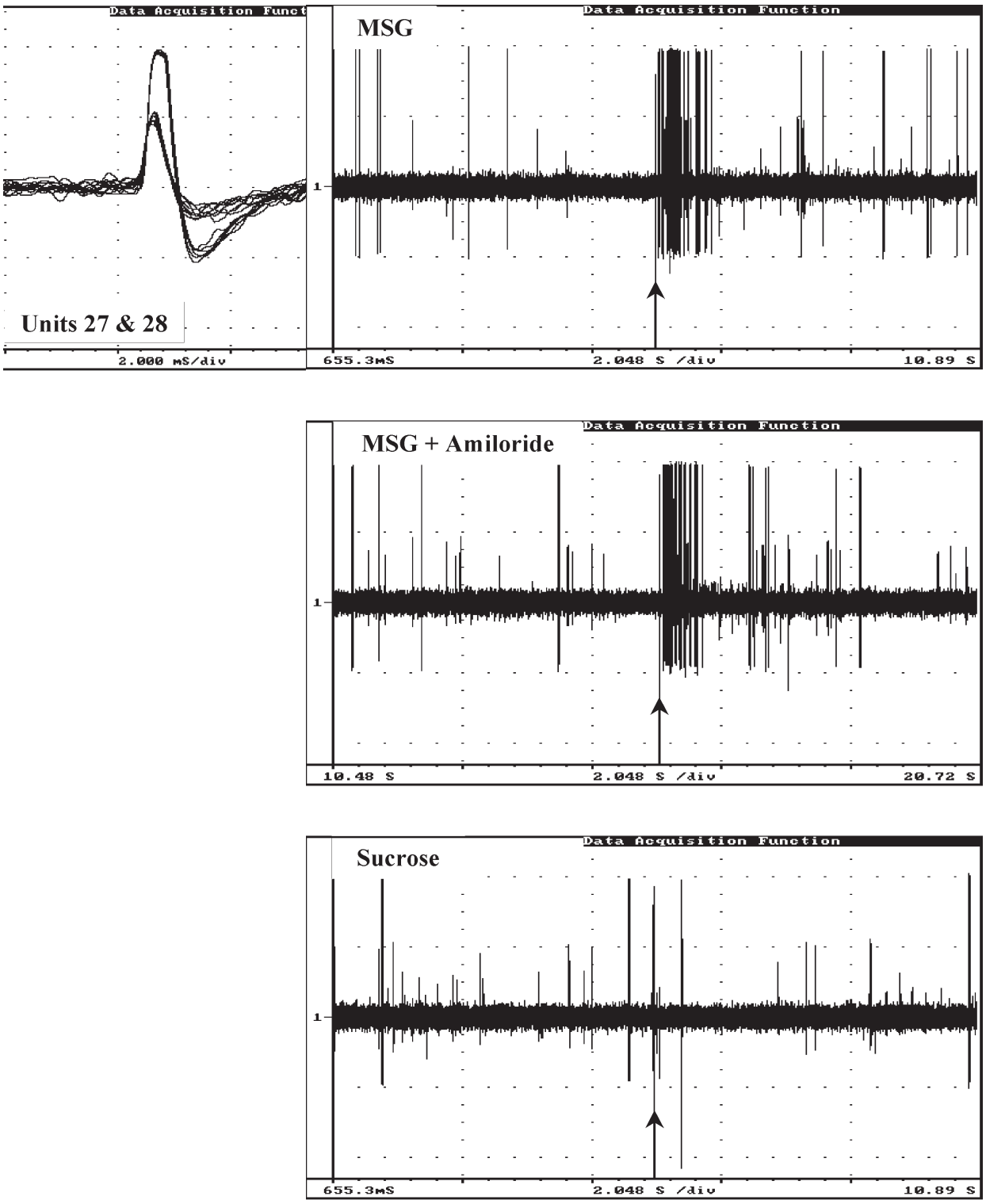

Figure 2 A 5 ms raw data trace showing the waveform discrimination of two single fibers and three 10 s traces showing responses of those fibers to MSG, MSG in amiloride and sucrose. The fibers were sensitive to MSG even after amiloride treatment and insensitive to sucrose. Shown in each response trace are two 5 s epochs, one before and one after stimulus application. Stimulus applications, marked with arrows, coincide with an artifact.

was considered stable if the steady-state responses to NH_4Cl that bracketed the series deviated by <15%. Multi-fiber responses were expressed relative to the mean of the two NH_4Cl standards bracketing the test concentration series. The relative response data for the sucrose and MSG concentration series and the MSG–sucrose binary mixtures were

analyzed using repeated-measures ANOVA with stimuli, concentration and presence of amiloride as factors in the analyses; *post hoc* tests used the Newman Keuls (NK) test.

In order to identify limits of mixture responses attributable to a single source (stimulus substitution) or two independent sources (response additivity; Hyman and Frank, 1980a), two derived data sets were also computed for MSG–sucrose mixtures with amiloride present. The data set for the stimulus substitution model was derived from CT responses to the sucrose concentration series (Figure 3). This model presumes that responses to glutamate–sucrose mixtures are due to activation of the same single pathway from sugar receptor to nerve. For each animal, response magnitudes for each MSG component were matched to sucrose response magnitudes. The sucrose concentration eliciting a response equal to the MSG response was then added to 300 mM and a predicted single-pathway mixture response was identified for the summed concentration. For example in Figure 3, the response to 0.1 M MSG matched the response to 0.14 M sucrose and thus, the response to 0.44 M sucrose was used for the stimulus substitution prediction. Therefore, predicted MSG–sucrose mixture responses for each animal were calculated by assuming that the glutamate component contributed to the CT response in the same manner as an equivalent concentration of sucrose (i.e. stimulus substitution). Calculations were performed in Microsoft Excel using two-point interpolations with a linear response axis and logarithmic concentration axis (Figure 3), methods similar to those used previously except that semi-logarithmic fits were used for data from each individual animal rather than power functions for mean data (Hyman and Frank, 1980a).

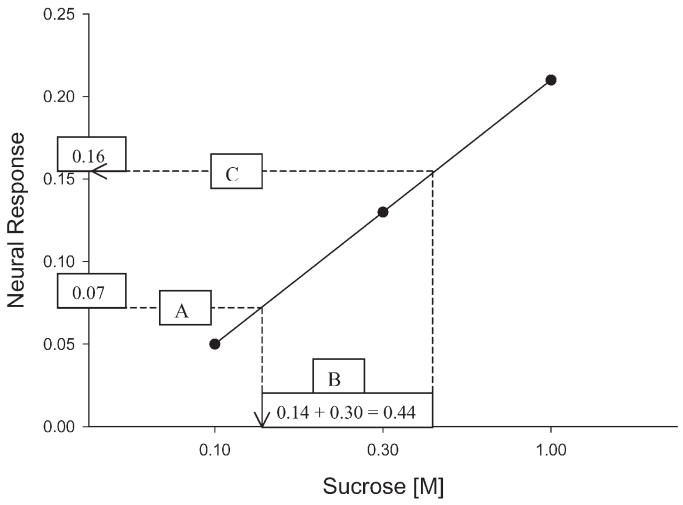

Figure 3 Determining the response predicted by stimulus substitution, a single-pathway mixture response. The figure illustrates three computation steps using neural response as a function of sucrose concentration for one animal. **(A)** The 0.1 M MSG component is determined equivalent to 0.14 M sucrose because they both elicit a response of 0.07. **(B)** The equivalent sucrose concentration for the mixture is 0.44, the sum of the sucrose concentration in the mixture (0.3 M) and the sucrose concentration equivalent to the MSG component. **(C)** The predicted stimulus substitution response to the mixture is 0.16.

The data set for the response additivity model was derived from CT responses to the mixture components. This model presumes that responses to glutamate–sucrose mixtures are due to activation of two separate pathways: one from sugar receptor to nerve and one from glutamate receptor to nerve. Predicted MSG–sucrose mixture responses for each animal were calculated by assuming that the sugar and the glutamate component contributed independently to the CT response. Thus, to obtain the predicted MSG–sucrose mixture response using response additivity the two CT responses to the individual components in each mixture were simply added together for each animal.

The mixture response models were evaluated along with the observed data using a repeated-measures ANOVA, with response (predicted by stimulus substitution, predicted by response additivity, and observed) and concentration as the within subjects factors in the analysis; *post hoc* tests used the NK test.

Single-fiber recordings

The set of fibers analyzed here are a subset (35%) of a larger fiber population and specifically chosen to help in the interpretation of whole-nerve responses to MSG–sucrose mixtures with amiloride present. Thus, the set included fibers that responded to sucrose and/or MSG, but the responses were *not* inhibited by amiloride. Inhibition by amiloride was defined as a >50% reduction in MSG response with 30 μM amiloride present.

A 5 s response measure was chosen for the single fibers because at least 5 s of spontaneous activity was recorded in all fibers prior to stimulus onset. A response was defined by the number of impulses in the first 5 s after stimulus onset and was compared to spontaneous activity, defined as the number of impulses in the 5 s immediately preceding stimulus onset. A fiber was considered stimulus activated if its response to the stimulus was greater than the fiber's mean 5 s spontaneous impulse rate plus 3 SE. Spontaneous rates and response rates to the various stimuli in subsets of fibers were evaluated by ANOVA or *t*-tests.

Results

Whole nerve

Amiloride (30 μM) suppressed CT responses to MSG and MSG–sucrose mixtures ($P < 0.01$) but did not significantly alter responses to sucrose, as revealed by analysis of the significant stimulus by amiloride treatment interaction [$F(2,14) = 31.45$, $P < 0.001$] (Figure 4). Analysis of the significant three-way, stimulus by concentration by amiloride treatment interaction [$F(4,28) = 38.56$, $P < 0.0001$] revealed that responses to MSG were greater than responses to sucrose at all concentrations before amiloride treatment; however, following amiloride treatment, MSG and sucrose

responses were similar at 30 and 100 mM, but responses to sucrose were slightly larger at 300 mM ($P < 0.05$). Whether amiloride was present or not, responses to the binary mixtures were greater than responses to either mixture component alone (Figure 5). Thus, inhibition was not observed for the MSG–sucrose mixtures.

Figure 6 illustrates the mean observed neural responses to mixtures of the three concentrations of MSG with 300 mM sucrose in the presence of amiloride and two hypothetical response functions, one predicted by response additivity (open squares) and the other predicted by stimulus substitution (open circles) (Hyman and Frank, 1080a). Analysis of the significant model by concentration interaction [$F(4,28) = 9.83$, $P < 0.0001$] revealed that the fit of the individual models to the observed mixture data changed as a function of MSG concentration. At all three concentrations of MSG, the observed responses were significantly greater than responses predicted by stimulus substitution ($P < 0.001$); thus none of the mixture responses could be attributed to a single process. At 30 mM MSG, a point where responses predicted by the two models were similar, observed responses to the mixture were significantly greater than the responses predicted by response additivity ($P < 0.001$). Predictions of the two models diverged at 100 mM MSG ($P < 0.001$), but both remained significantly smaller than observed mixture responses ($P < 0.05$ for the response additivity model and $P < 0.001$ for the stimulus substitution model). At 300 mM MSG, observed mixture responses were similar to predictions for response additivity. Thus, at low MSG concentrations, MSG–sucrose mixtures elicited responses above the range predicted for two independent processes, suggesting synergy or enhancement. However, as more MSG was added to the mixture, MSG–sucrose mixture responses more closely resembled independent processes.

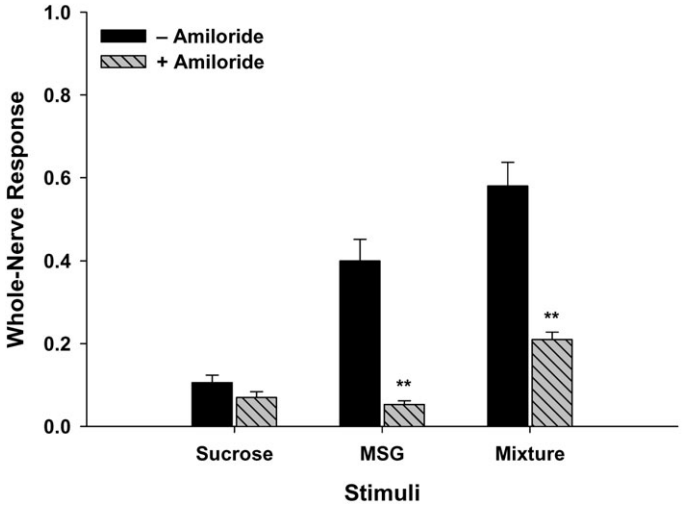

Figure 4 Mean (± SEM) relative neural responses to sucrose, MSG and the MSG–sucrose mixture with (+) and without (-) 30 μM amiloride treatment ($n = 8$). Responses were averaged across concentrations for each stimulus. All mixtures contained 300 mM sucrose. Stimuli applied following amiloride treatment contained 30 μM amiloride. Responses significantly smaller in the presence of amiloride are indicated: $^{**}P < 0.01$.

Single fibers

Figure 7 illustrates response profiles for 14 CT fibers that responded to 300 mM sucrose and/or 100 mM MSG, but were not inhibited by 30 μM amiloride. All seven sucrose-sensitive fibers also responded to MSG. The responses to sucrose, MSG and MSG plus amiloride all exceeded the

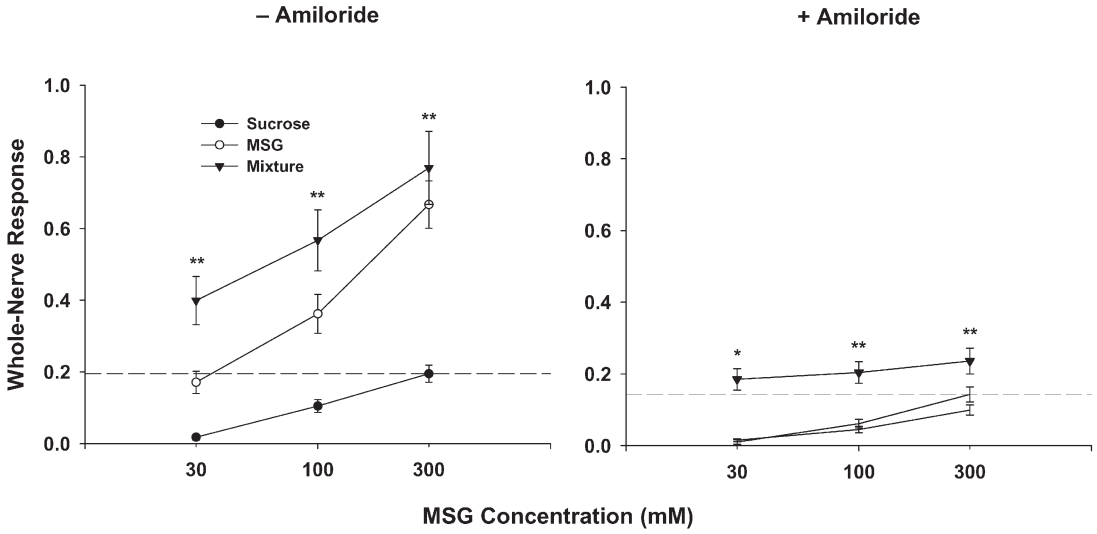

Figure 5 Mean (± SEM) relative neural response to sucrose, MSG and the MSG–sucrose mixture before (–) and after (+) treating the tongue with 30 μM amiloride (n = 8). Each mixture contained 300 mM sucrose and the dashed line in each graph indicates the mean response to 300 mM sucrose presented alone. Stimuli applied following amiloride treatment contained 30 μM amiloride. Responses to mixtures were greater than responses to either mixture component, $^{*}P < 0.05$; $^{**}P < 0.01$.

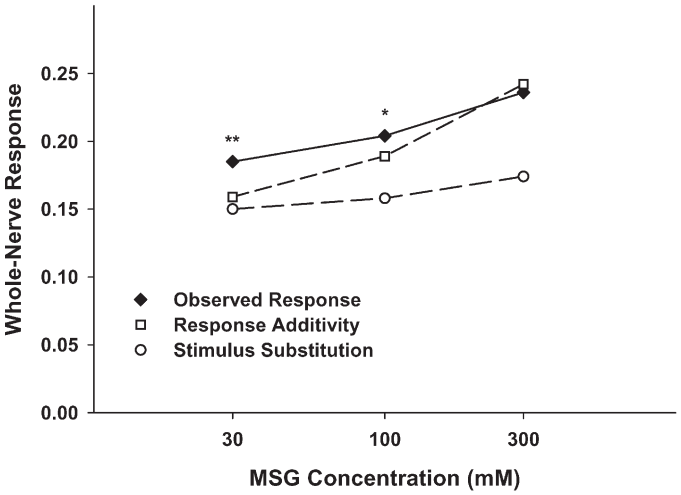

Figure 6 Mean response concentration functions for the two mixture response models and the observed mixture responses after treating the tongue with 30 μM amiloride. Shown are measured responses for mixtures of 300 mM sucrose with the 3 concentrations of MSG (closed diamonds), responses predicted by response additivity (open squares) and responses predicted by stimulus substitution (open circles). Responses to mixtures greater than predictions for response additivity are indicated: $*P < 0.05$; $**P < 0.01$.

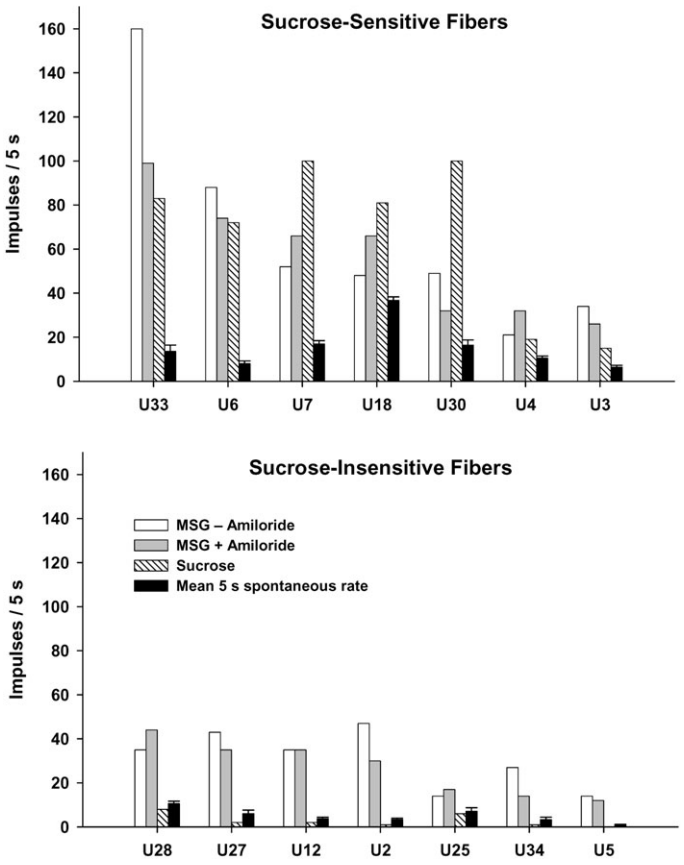

Figure 7 Response profiles for 14 CT fibers sensitive to 100 mM MSG after amiloride treatment. Fibers were divided into two groups based on sensitivity to 300 mM sucrose and were arranged from left to right in descending order according to MSG with (+) amiloride sensitivity. Raw data traces for fibers U27 and U28 are illustrated in Figure 2.

average spontaneous rate by >3 SE and impulse rates to the three stimuli were statistically equivalent in sucrose-sensitive fibers. The other seven fibers were sucrose-insensitive; the responses to MSG and MSG plus amiloride met the same response criterion. In these fibers, response rates to the two presentations of MSG (+ amiloride, – amiloride) were statistically equivalent. There were no fibers that responded to 300 mM sucrose that did not also respond to 100 mM MSG (+ amiloride).

On average across the three stimuli, sucrose-sensitive fibers responded at higher rates than sucrose-insensitive fibers [$F(1,12) = 12.12$, $P < 0.005$]. Mean (± SEM) response rates to MSG with amiloride (56 ± 10 versus 27 ± 4.7 impulses), $P = 0.005$; MSG without amiloride (65 ± 18 versus 31 ± 4.9), $P = 0.007$; as well as the mean response rates to sucrose (67 ±1 4 versus 3 ± 1.1), $P = 0.0001$, were significantly greater in sucrose-sensitive fibers than sucrose-insensitive fibers. Sucrose-sensitive fibers also had significantly higher average spontaneous rates than sucrose-insensitive fibers (16 ± 3.8 versus 5 ± 3.8 impulses), $t(12) = 2.60$, $P = 0.05$. Thus, sucrose-sensitive fibers were more reactive as a group than sucrose-insensitive fibers.

In the whole nerve, fibers responding to MSG and sucrose would likely contribute to MSG–sucrose mixture responses as a single source and conform to the stimulus substitution model, as do mixtures of two sweeteners (Hyman and Frank, 1980a). However, the MSG-responsive fibers that were insensitive to sucrose would contribute an additional independent, additive, component to the MSG–sucrose mixture responses of the whole nerve. Thus, the fact that MSG activates sucrose-sensitive and sucrose-insensitive CT fibers helps explain the failure of the stimulus substitution model for MSG–sucrose mixtures in amiloride. However, based solely on the effects of the individual components on the two groups of CT neural units, the observed mixture response would have been expected to fall between stimulus substitution and response additivity.

Discussion

Behavioral and neurophysiological data support the idea that the taste of MSG has a complex quality attributable in part to the sodium cation and in part, to the glutamate anion in rodents such as rats, hamsters and mice (Yamamoto *et al.*, 1988, 1991; Ninomiya and Funakoshi, 1989a,b; Hettinger and Frank, 1990; Hill *et al.*, 1990; Stapleton *et al.*, 2002). To probe the source of glutamate's effect on rat CT responses, MSG–sucrose mixtures were studied after blocking ENaC with amiloride. Multiple sources contributed to the CT response to MSG in the presence of amiloride.

Stimulation with MSG, like quinine, is associated with decreases in intracellular cyclic adenosine monophosphate (cAMP) concentration (Yan *et al.*, 2001; Abaffy *et al.*, 2003),

whereas sucrose stimulation is associated with increases in cAMP concentration (Striem *et al.*, 1989, 1991). If these intracellular responses occurred in the same cell, responses to MSG or quinine might well be antagonistic to sucrose and inhibit sucrose responses in taste receptor cells that drive the CT; responses to MSG–sucrose mixtures would be smaller than responses to sucrose alone. However, this was not the case; unlike quinine (Formaker and Frank, 1996; Formaker *et al.*, 1997), glutamate's effects did not appear to include inhibition of CT responses to sucrose, whether amiloride was present or not.

Because similarities are perceived in the tastes of sucrose and MSG in the presence of amiloride by rats, another possibility was that glutamate, like sugars and some other amino acids, would activate taste receptors that respond to sucrose, such as the heterodimeric T1r2-3 complex (Yamamoto *et al.*, 1991; Li *et al.*, 2002; Nelson *et al.*, 2002; Stapleton *et al.*, 2002; Damak *et al.*, 2003; Zhao *et al.*, 2003). Credence is given this hypothesis by the occurrence of glutamate responses in all sucrose-sensitive CT nerve fibers in our sample. However, evidence that T1r2–3 is not sensitive to glutamate (Li *et al.*, 2002) argues against this possibility. The current results could also be explained by postulating the coexistence of candidate T1r2–3 sugar and T1r1–3 glutamate receptors on the same taste-receptor cell (TRC), but experimental evidence in mice argues against this possibility (Nelson *et al.*, 2001). Finally, convergence of a TRC expressing T1r2–3 and another, independent, TRC expressing T1r1–3 onto a single CT nerve fiber could also explain the current results.

CT responses to MSG–sucrose mixtures were all larger than responses predicted by stimulus substitution. Stimulus substitution would prevail if two mixture components were both ligands for the same receptor(s) and not ligands for other receptors. The possibility that more than one receptor is activated by MSG presented with amiloride is supported by the single-fiber results showing there are two functional classes of amiloride-insensitive, glutamate-sensitive, CT fibers, one that responds to sucrose and another that does not. Thus, MSG in the presence of amiloride activates a class of CT neural units beyond those units sensitive to sucrose. Those units may be driven by TRCs expressing glutamate receptors and not sugar receptors, a postulate supported by experimental evidence (Nelson *et al.*, 2001).

There were no rat CT neurons in the current data set that responded to sucrose that did not also respond to glutamate. It would be interesting to know if the highly reactive sucrose-best neural units of the rat greater superficial petrosal nerve (GSP) also respond to MSG. Rat GSP units are much more responsive to sucrose than rat CT units, which are rarely sucrose-best, unlike mouse and hamster CT units (Hyman and Frank, 1980a,b; Frank *et al.*, 1983; Sollars and Hill, 1998, 2001). Compared to the CT, GSP neural units may be more extensively and specifically activated by T1r2–3 sugar receptors in rats.

If glutamate were a ligand for two or more independent receptors, e.g. the candidate taste-mGluR4, heterodimeric T1r1–3 and T1r2–3, for which sucrose also is a ligand, the CT response to MSG–sucrose mixtures (in amiloride) would be greater than the prediction for stimulus substitution (i.e. one receptor for both ligands) but less than the prediction for response additivity (i.e. separate receptors for each ligand). However, CT responses to the mixture of 300 mM sucrose with 300 mM MSG equaled the prediction for response additivity; furthermore, CT responses to mixtures of sucrose with 30 and 100 mM MSG exceeded response additivity predictions. These results indicate mixture responses cannot be explained on the basis of component responses alone and suggest a synergistic mixture interaction between sucrose and MSG. A potential source of that synergy argues against a model based on mouse data that has sugar and glutamate receptors in separate TRCs operating independently (Zhao *et al.*, 2003); this difference may reflect species variation.

Synergy was recently observed in a subset of sucrose-insensitive rat CT fibers when the tongue was stimulated with a mixture of 5 mM L-amino-4-phosphonobutyrate (LAP4) and 100 mM sucrose (Sako *et al.*, 2003). LAP4 is an agonist for the type 4 metabotropic glutamate receptor (mGluR4); a truncated version, taste-mGluR4, is found in taste tissue (Chaudhari *et al.*, 2000; Sako *et al.*, 2003). No LAP4-sucrose synergy was seen in sucrose-sensitive CT fibers. Thus, it is possible that the sucrose-insensitive, glutamate-responsive, CT fibers would show synergy when the MSG–sucrose mixture is applied to the tongue; conversely, sucrose and glutamate sensitive fibers would not. If sucrose-sensitive and sucrose-insensitive CT units were activated by identical glutamate receptors, both groups of units would likely show synergy to the sucrose-glutamate mixture. Thus, glutamate is probably a ligand for more than one taste receptor in rats.

In summary, analysis of MSG–sucrose mixtures reveals that the sources of MSG-elicited CT responses in rats are multiple, even in an amiloride-inhibited environment. Glutamate-sensitive CT neural units that also respond to sucrose and their associated receptors may generate the portion of the glutamate response that is the basis for a sucrose-glutamate perceptual similarity (Yamamoto *et al.*, 1991; Chaudhari *et al.*, 1996; Stapleton *et al.*, 1999; Heyer *et al.*, 2003). However, less-reactive sucrose-insensitive CT neural units and their associated receptors may respond synergistically to glutamate–sucrose mixtures and dominate mixture responses yielding levels of activity that exceed predictions consistent with two independent sources. These latter neural units may contribute to the unique taste of MSG in rats.

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References

Abaffy, T., Trubey, K.R. and **Chaudhari, N.** (2003) *Adenylyl cyclase expression and modulation of cAMP in rat taste cells*. Am. J. Physiol. Cell Physiol., 284, C1420–C1428.

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

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

Damak, S., Rong, M., Yasumatsu, K., Kokrashvili, Z., Varadarajan, V., Zou, S., Jiang, P., Ninomiya, Y. and **Margolskee, R.F.** (2003) *Detection of sweet and umami taste in the absence of taste receptor T1r3*. Science, 301, 850–853.

DeSimone, J.A., Callaham, E.M. and **Heck, G.L.** (1995) *Chorda tympani taste response of rat to hydrochloric acid subject to voltage-clamped lingual receptive field*. Am. J. Physiol., 268, C1295–C1300.

Formaker, **B.K.** and **Frank, M.E.** (1996) *Responses of the hamster chorda tympani nerve to binary component taste stimuli: evidence for peripheral gustatory mixture interactions*. Brain Res., 727, 79–90.

Formaker, **B.K.** and **Hill, D.L.** (1988) *An analysis of residual NaCl taste response after amiloride*. Am. J. Physiol., 255, 1002–1007.

Formaker, B.K., MacKinnon, B.I., Hettinger, T.P. and **Frank, M.E.** (1997) *Opponent effects of quinine and sucrose on single-fiber taste responses of the chorda tympani nerve*. Brain Res., 772, 239–242.

Frank, M.E., Contreras, R.J. and **Hettinger, T.P.** (1983) *Nerve fibers sensitive to ionic taste stimuli in chorda tympani of the rat*. J. Neurophysiol., 50, 941–960.

Halpern, **B.P.** (2002) *What's in a name? Are MSG and umami the same?* Chem. Senses, 27, 845–846.

Hettinger, **T.P.** and **Frank, M.E.** (1990) *Specificity of amiloride inhibition of hamster taste responses*. Brain Res., 513, 24–34.

Hettinger, T.P., Frank, M.E. and **Myers, W.E.** (1996) *Are the tastes of polycose and monosodium glutamate unique?* Chem. Senses, 21, 341–347.

Heyer, B.R., Taylor-Burds, C.C., Tran, L.H. and **Delay, E.R.** (2003) *Monosodium glutamate and sweet taste: generalization of conditioned taste aversion between glutamate and sweet stimuli in rats*. Chem. Senses, 28, 631–641.

Hill, **D.L.** and **Bour, T.C.** (1985) *Addition of functional amiloride-sensitive components to the receptor membrane: a possible mechanism for altered taste responses during development*. Brain Res., 352, 310–313.

Hill, D.L., Formaker, B.K. and **White, K.S.** (1990) *Perceptual characteristics of the amiloride-suppressed sodium chloride taste response in the rat*. Behav. Neurosci., 104, 734–741.

Hyman, **A.M.** and **Frank, M.E.** (1980a) *Effects of binary taste stimuli on the neural activity of the hamster chorda tympani*. J. Gen. Physiol., 76, 125–142.

Hyman, **A.M.** and **Frank, M.E.** (1980b) *Sensitivities of single nerve fibers in the hamster chorda tympani to mixtures of taste stimuli*. J. Gen. Physiol., 76, 143–173.

Ikeda, **K.** (2002) *New seasonings*. Chem. Senses, 27, 847–849.

Kawamura, **Y.** and **Kare, M.R.** (1987) Umami: A Basic Taste. Marcel Dekker, Inc, New York.

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

Lindemann, B., Ogiwara, Y. and **Ninomiya, Y.** (2002) *The discovery of umami*. Chem. Senses, 27, 843–844.

Lundy, **R.F.** and **Contreras, R.J.** (1997) *Temperature and amiloride alter taste nerve responses to Na^+, K^+, and NH_4^+ salts in rats*. Brain Res., 744, 309–317.

Nelson, G., Hoon, M.A., Chandrashekar, J., Zhang, Y., Ryba, N.J. and **Zuker, C.S.** (2001) *Mammalian sweet taste receptors*. Cell, 106, 381–390.

Nelson, G., Chandrashekar, J., Hoon, M.A., Feng, L., Zhao, G., Ryba, N.J. and **Zuker, C.S.** (2002) *An amino-acid taste receptor*. Nature, 416, 199–202.

Ninomiya, **Y.** and **Funakoshi, M.** (1988) *Amiloride inhibition of responses of rat single chorda tympani fibers to chemical and electrical tongue stimulations*. Brain Res., 451, 319–325.

Ninomiya, **Y.** and **Funakoshi, M.** (1989a) *Behavioral discrimination between glutamate and the four basic taste substances in mice*. Comp. Biochem. Physiol. A, 92, 365–370.

Ninomiya, **Y.** and **Funakoshi, M.** (1989b) *Peripheral neural basis for behavioural discrimination between glutamate and the four basic taste substances in mice*. Comp. Biochem. Physiol. A, 92, 371–376.

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

Sako, N., Tokita, K., Sugimura, T. and **Yamamoto, T.** (2003) *Synergistic responses of the chorda tympani to mixtures of umami and sweet substances in rats*. Chem. Senses, 28, 261–266.

Schiffman, S.S., Mcelroy, A.E. and **Erickson, R.P.** (1980) *The range of taste quality of sodium salts*. Physiol. Behav., 24, 217–224.

Sollars, **S.I.** and **Hill, D.L.** (1998) *Taste responses in the greater superficial petrosal nerve: substantial sodium salt and amiloride sensitivities demonstrated in two rat strains*. Behav. Neurosci., 112, 991–1000.

Sollars, **S.I.** and **Hill, D.L.** (2001) *Neurophysiological taste responses of single geniculate ganglion cells recorded* in vivo *in the rat*. Chem. Senses, 26, 1084.

Spector, A.C., Guagliardo, N.A. and **St John, S.J.** (1996) *Amiloride disrupts NaCl versus KCl discrimination performance: implications for salt taste coding in rats*. J. Neurosci., 16, 8115–8122.

Stapleton, J.R., Roper, S.D. and **Delay, E.R.** (1999) *The taste of monosodium glutamate (MSG),* L*-aspartic acid, and* N*-methyl-*D*-aspartate (NMDA) in rats: are NMDA receptors involved in MSG taste?* Chem. Senses, 24, 449–457.

Stapleton, J.R., Formaker, B.K., Roper, S.D. and **Frank, M.E.** (2000) *Responses of the rat chorda tympani to mixtures of MSG and sucrose in the presence of amiloride*. Chem. Senses, 25, 681.

Stapleton, J.R., Luellig, M., Roper, S.D. and **Delay, E.R.** (2002) *Discrimination between the tastes of sucrose and monosodium glutamate in rats*. Chem. Senses, 27, 375–382.

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

Striem, B.J., Naim, M. and **Lindemann, B.** (1991) *Generation of cyclic AMP in taste buds of the rat circumvallate papilla in response to sucrose*. Cell. Physiol. Biochem., 1, 46–54.

Yamamoto, **T.** and **Kawamura, Y.** (1974) *An off-type response of the chorda tympani nerve in the rat*. Physiol Behav., 13, 239–243.

Yamamoto, T., Matsuo, R., Kiyomitsu, Y. and **Kitamura, R.** (1988) *Taste effects of 'umami' substances in hamsters as studied by electrophysiological and conditioned taste aversion techniques*. Brain Res., 451, 147–162.

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

Yan, W, Sunavala,G., Rosenzweig,S., Dasso,M., Brand,J.G. and **Spielman,A.I.** (2001) *Bitter taste transduced by PLC-beta(2)-dependent rise in IP(3) and alpha-gustducin-dependent fall in cyclic nucleotides*. Am. J. Physiol. Cell. Physiol., 280, C742-C751.

Zhao, G.Q., Zhang, Y., Hoon, M.A., Chandrashekar, J., Erlenbach, I., Ryba, N.J.P. and **Zuker, C.S.** (2003) *The receptors for mammalian sweet and umami taste*. Cell, 115, 255–266.

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