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Monosodium Glutamate and Sweet Taste: Generalization of Conditioned Taste Aversion between Glutamate and Sweet Stimuli in Rats

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Abstract

Even though monosodium glutamate (MSG) is a prototypical umami substance, previous studies reported that a conditioned taste aversion (CTA) to MSG, mixed with amiloride to block the taste of sodium, generalizes to sucrose. These findings suggest that the taste of glutamate mimics the taste of sucrose and raise the question of whether glutamate has a broadly tuned sweet taste component. To test this hypothesis, CTA experiments were conducted to test for generalization between MSG and several sweet stimuli: sucrose, glucose, maltose, saccharin and SC-45647. Strong bidirectional generalization was seen between MSG mixed with amiloride and sucrose, glucose, saccharin and SC-45647. Weak generalization was seen between MSG and maltose, and sucrose and maltose. None of the CTAs generalized to NMDA. These findings support the hypothesis that the taste of MSG has broadly tuned, sweet-like characteristics, possibly due to the convergence of afferent signals for MSG, natural sugars and artificial sweeteners.

Key words: conditioned taste aversion, monosodium glutamate, sweet stimuli, sweet taste, umami

Introduction

Many substances produce a taste sensation but there are only a few primary tastes: sour, salty, bitter and sweet. Although controversial, glutamate is also said to possess a unique taste quality known as 'umami', which is thought to be distinct from sweet, sour, salty and bitter (Yamaguchi, 1967). Each basic taste influences ingestive behavior and/or signals a general food type. Sweet, for example, is generally associated with carbohydrates, and sucrose is generally accepted as the prototypical sweet substance. The prototypical stimulus that elicits an umami taste is monosodium glutamate (MSG), which is a naturally occurring amino acid that, in small quantities, has long been incorporated into Asian cuisine to enhance flavor (Maga, 1983). It is also a natural constituent of many protein-rich food items such as meats, cheese and some vegetables. The ability of an organism to detect glutamate is important because its taste signals the presence of dietary protein, and it can increase the palatability of food and, thus, food intake.

Even though humans perceive the taste of MSG as umami (Kurihara and Kashiwayanagi, 1998), under certain conditions rats perceive the taste of MSG as similar to that of sucrose. If a taste aversion is conditioned (CTA) in rats to MSG mixed with amiloride (a Na^+ channel blocker that reduces the Na^+ component of MSG taste), this CTA generalizes to sucrose (Yamamoto *et al.*, 1991; Chaudhari *et al.*, 1996; Stapleton *et al.*, 1999). This finding is curious for several reasons. Behavioral and molecular evidence (Chaudhari *et al.*, 1996, 2000; Stapleton *et al.*, 1999; Delay *et al.*, 2000) indicates that in rats glutamate activates a novel taste variant of a G protein-coupled class III metabotropic glutamate receptor (mGluR4). Chaudhari *et al.* (2000) cloned a taste-mGluR4 receptor that is identical to the brain mGluR4 except the n-terminus of the taste-mGluR4 is truncated and the receptor has a lower affinity for glutamate. Activation of taste-mGluR4 receptors down-regulates cAMP and produces an increase in activity in afferent fibers (Chaudhari and Roper, 1998). Other researchers have reported that glutamate and other substances that elicit an umami taste in humans also activate a general amino acid receptor, T1R1+T1R3 (Li *et al.*, 2002; Nelson *et al.*, 2002) and that MSG may activate an IP_3 second-messenger pathway in taste receptor cells (Nakashima and Ninomiya, 1998). In contrast, stimuli that elicit a sweet sensation in humans activate a different heterodimeric receptor (T1R2 + T1R3) that is not activated by umami substances (Li *et al.*, 2002). In spite of apparent differences in afferent mechanisms activated by MSG and sucrose, the CTA data suggest that to rats, MSG mixed with amiloride mimics the taste of sucrose and, thus, raise the question of whether MSG also

mimics the tastes of other substances that elicit a sweet sensation.

To address this question, we conducted CTA experiments to determine the degree of cross-generalization of a CTA between MSG and several natural sugars and artificial sweeteners. These substances elicit a sweet sensation in humans (Schiffman *et al.*, 1981) and are preferred by rats (Richter and Campbell, 1940; Noma *et al.*, 1971; Smith and Sclafani, 2002). For convenience, these substances hereafter will be referred to as sweet-eliciting or sweet substances. We selected several stimuli that are thought to elicit sweet sensations by activating different G protein-coupled second messenger systems within taste receptor cells. Sucrose and presumably other natural sugars are believed to activate a G protein-coupled receptor that increases cAMP within receptor cells (Bernhardt *et al.*, 1996). Artificial sweeteners, on the other hand, appear to activate a G protein-coupled receptor that increases IP_3 (Bernhardt *et al.*, 1996; Nakashima and Ninomiya, 1998). The strength of CTA procedures is that an aversion learned for one taste substance will cause a subject to avoid other stimuli that elicit a similar taste. Moreover, the greater the similarity between the tastes of two substances, the more the CTA will generalize between the two substances. If glutamate elicits a basic sweet sensation to rats, then a CTA should generalize between MSG mixed with amiloride and natural sugars and artificial sweeteners. However, if MSG elicits a taste that is unlike the tastes of either natural sugars or artificial sweeteners, then a CTA to MSG with amiloride should not generalize to all sweet substances and visa versa.

Experiment 1

The first experiment was conducted to determine concentrations of maltose, glucose, sodium saccharin and SC-45647 to use for generalization testing in the cross-generalization experiment.

Materials and methods

Subjects

Sixty-two male albino Sprague–Dawley rats were obtained from Harlan Sprague–Dawley (Indianapolis, IN). At the beginning of the experiment all animals were 90–120 days old, weighed 310–450 g and were housed individually with Purina Lab chow available *ad libitum*. Beginning 14 days before the experiment, the rats were placed on a 21.5 h water deprivation schedule that was maintained throughout the experiment. The colony lighting was regulated according to a 12 h light/dark schedule with the lights turned on at 7:45 a.m. All testing took place during the light portion of the cycle and each rat was tested at the same time each day.

Apparatus

Each test station housed a computer controlled Davis MS80 Lickometer system (DiLog Instruments, Tallahassee, FL) with an enclosed Plexiglas operant chamber with a metal grid floor. An oval-shaped opening covered by a computer-operated metal shutter was located at one end of the chamber. Eight stimulus tubes with lick spouts were mounted on a moveable platform behind the oval-shaped opening. The rats had access to a taste solution when the shutter was opened. During a trial, the tube spout was 3 mm behind the center of the oval-shaped opening. A lick was counted when a rat licked from the metal spout and completed a 64 nA contact current. To reduce olfactory cues, air flowed into the operant chamber from a tube mounted on the far wall of the chamber and exited the chamber through the oval shaped opening. The walls and ceiling of each test station were constructed of wood. A dark blue felt curtain covered the front of the test station to reduce distracting visual and auditory cues. Masking noise (75 ± 5 dB; Radio Shack Sleep Machine) was present throughout all sessions.

Procedure

All behavioral training and testing were carried out in the Davis Lickometer for seven consecutive days as follows. During the first three days, rats were trained to drink deionized water from the Lickometer. Each session consisted of 32 trials and lasted 15–20 min. The rat initiated a trial by making contact with the delivery spout. Licks emitted during each 10 s trial were counted. Rats were given up to 60 s to begin a trial before the shutter closed and the next stimulus was presented. A 5 s intertrial interval followed each trial. One hour after the end of the session, each rat was given access to a water bottle for 1 h.

On the fourth day, 16 rats were randomly assigned (8 to an experimental group and 8 to a control group) to each conditioned stimulus (CS) except SC-45647. SC-45647, a potent artificial sweetener thought to activate similar transduction mechanisms as saccharin (Nofre *et al.*, 1990; Bernhardt *et al.*, 1996; Danilova *et al.*, 1998; Varkevisser and Kinnamon, 2000), was tested with 14 rats. During the conditioning session, the CS was randomly presented 16 times amidst water trials. CS and water solutions contained 30 μM of amiloride to reduce potentially confounding taste elicited by Na^+ (Heck *et al.*, 1984; Geran and Spector, 2000). At this concentration amiloride is not recognizable to rats (Markison and Spector, 1995). Immediately after drinking the CS, the rats assigned to the experimental group received i.p. injections of 0.3 M LiCl (127 mg/kg, 1 ml/100 g body wt) as an unconditioned stimulus (US) to induce gastric distress and thus a conditioned aversion to the taste stimulus (Nachman and Ashe, 1973; Spector and Grill, 1988). The rats assigned to the control group received i.p. injections of 0.15 M NaCl (1 ml/100 g body wt) as the US. CS concentrations that produced comparable reliability of conditioning were determined from pilot studies and were as follows: 150 mM glucose, 100 mM maltose, 1.25 mM saccharin and 0.05 mM SC-45647. The two days following conditioning

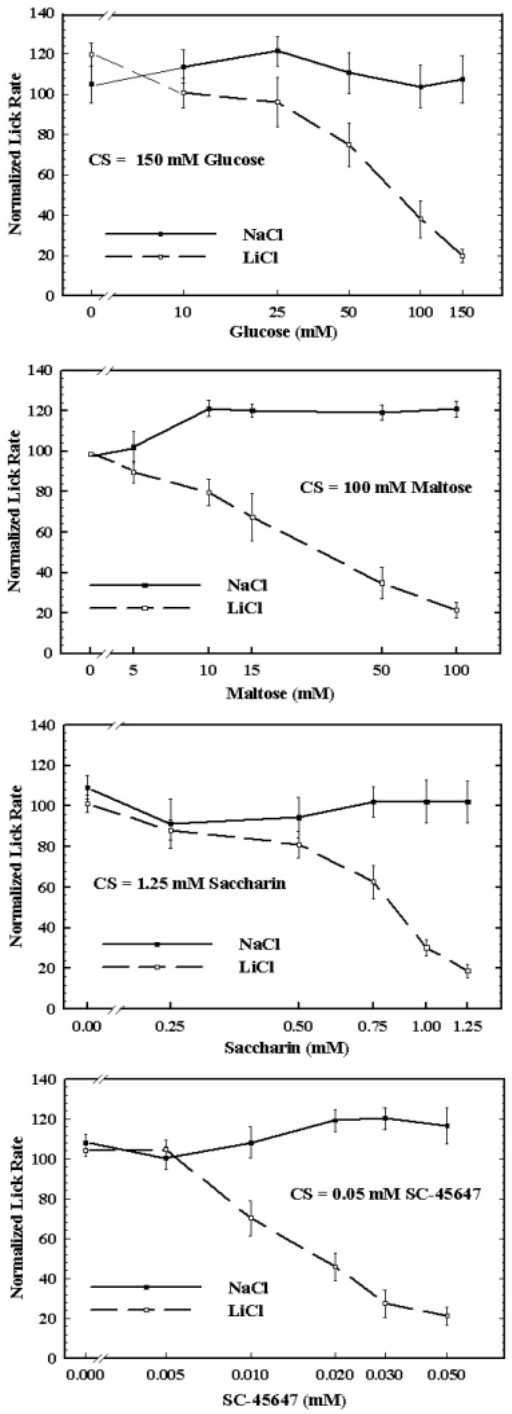

Figure 1 Mean (±SEM) normalized lick rates for several concentrations of four sweet substances after being conditioned with either NaCl (control, filled squares) or LiCl (experimental, open squares) injections to induce a taste aversion. The CS concentration was 150 mM glucose (top), 100 mM maltose (second from top), 1.25 mM saccharin (third from top), and 0.05 mM SC-45647 (bottom). To normalize lick rates, the mean lick rate for each test stimulus was divided by the mean lick rate for all water presentations in that session, then multiplied by 100. The ordinate shows the normalized lick rates and the abscissa shows the concentrations of the test solutions. In this and in all subsequent figures, amiloride was present in all solutions to reduce Na^+ taste.

were 'washout' days in which the rats were presented only deionized water.

On the seventh day, a concentration gradient for the strength of each taste aversion was determined with five concentrations of the CS, each presented twice during the single session. The glucose gradient was established using 10, 25, 50, 100 and 150 mM glucose during the test session. For maltose the gradient was determined with 5, 10, 15, 50 and 100 mM solutions. The gradient for saccharin was established using 0.25, 0.50, 0.75, 1.0 and 1.25 mM. To establish the gradient for SC-45647, rats were tested with 0.005, 0.01, 0.02, 0.03 and 0.05 mM SC-45647. All rats were also tested with 25 mM KCl to determine if the rats were avoiding all detectable taste stimuli and with 100 mM sucrose to determine if the CTA generalized to a prototypical substance that elicits a sweet taste. The order of stimulus presentation to each rat was randomized using a Latin square. Stimuli were separated by 1–3 water trials. Suppression was measured as a percentage of licks for a stimulus relative to licks for water. Thus, lick rates below 100% indicate avoidance of a stimulus relative to water, and lick rates by LiCl-conditioned rats that are less than those seen for NaCl-conditioned rats indicate CTA.

Results and discussion

Before any analyses were performed, the data for each animal were normalized by dividing the average licks for each stimulus by the average number of licks for water trials, then multiplying by 100. To generate a score for a 'zero' concentration for each rat that was comparable to the other taste stimuli, all series of two or more consecutive water trials were identified and two trials from these series were randomly selected (the first trial of each series was excluded). The lick rates for these two trials were normalized and treated like all other taste stimuli.

A 2 (US) × 8 (taste stimuli) analysis of variance (ANOVA) for mixed designs was used to analyze the normalized lick rates for each substance (see Figure 1). Each of these ANOVAs detected significant effects due to the US conditions (NaCl or LiCl) [$F(1,14) = 32.64$ or greater, $P < 0.001$], all eight of the taste solutions [$F(7,98) = 6.67$ or greater, $P < 0.001$], and the interaction [$F(7,98) = 7.52$ or greater, $P < 0.001$] between these two variables. Similar effects were found for SC-45647 [US: $F(1,12) = 108.55$, $P < 0.001$; taste stimuli: $F(7,84) = 14.02$, $P < 0.001$; interaction: $F(7,84) = 39.94$, $P < 0.001$]. Simple effects tests were then conducted on each set of data to determine which stimulus solutions were avoided by LiCl-injected rats compared to the NaCl rats. These tests indicated that the lowest concentrations for which the LiCl-conditioned rats showed significantly [$F(1,14) = 4.66$ or greater, $P < 0.05$ or less] lower lick rates than NaCl-injected rats were: (i) glucose = 25 mM, (ii) maltose = 10 mM, (iii) saccharin = 0.75 mM and (iv) SC-45647 = 0.01 mM. Moreover, as seen in Table 1, lick rates for 25 mM KCl were not significantly altered by the CTA to

Table 1 Mean (SEM) normalized lick rates for control stimuli in experiment 1 after injections of NaCl or LiCl

CS	US	KCl	Sucrose
Glucose	NaCl	104.59 (5.17)	121.82 (12.48)
	LiCl	81.58 (14.12)	27.51 (5.86)***
Maltose	NaCl	105.95 (4.56)	90.95 (10.14)
	LiCl	91.05 (4.83)	58.81 (12.00)
Saccharin	NaCl	97.04 (8.17)	105.83 (9.63)
	LiCl	79.91 (7.54)	21.54 (2.55)***
SC-45647	NaCl	90.24 (6.2)	122.71 (6.35)
	LiCl	104.89 (9.13)	26.85 (3.71)***

***$P < .005$; all other comparisons $P > 0.05$.

any of the sweet substances but the intake of 100 mM sucrose was decreased significantly by the CTA to every sweet substance [$F(1,14) = 13.64$ or greater, $P < 0.005$] except maltose ($P < 0.10$).

Animals readily developed a CTA to each CS after LiCl injections. In each case, the magnitude of the CTA increased as the concentration increased. None of the CTAs generalized to KCl and, except maltose, all CTAs generalized to sucrose. These results indicate that amount of suppression of lick rates was related to the perceived intensity of the qualities of the respective CS and not simply due to the detection of any taste sensation.

Experiment 2

The second experiment was conducted to determine whether a CTA to MSG would generalize to natural sugars (sucrose, glucose, maltose) or artificial sweeteners (sodium saccharin, SC-45647) and, conversely, whether a CTA to each of these substances would generalize to MSG.

Materials and methods

Subjects

Subjects were 160 naïve male, albino rats of the same characteristics as those obtained for experiment 1. Housing and animal care were also the same as experiment 1.

Apparatus and procedures

The apparatus and general procedures of conditioning and testing were the same as in experiment 1 with the following changes. Thirty-two rats were used to test for generalization between each sweet substance and MSG. On the fourth day, half of these rats were conditioned with 100 mM MSG and the other half were conditioned with the sweet substance. Each of these groups was further subdivided into two equal sized groups, one conditioned with LiCl injections and the other with NaCl injections. Assignment to each condition was random. The concentrations of each CS were as follows: MSG = 100 mM, glucose = 150 mM, maltose = 100 mM, saccharin = 1.25 mM, and SC-45647 = 0.05 mM.

During generalization testing on day 7, two concentrations of the CS were presented to the rats to determine the strength of conditioning. Three concentrations of each test substance were presented to determine if the CTA generalized to the opposite substance. The CS and test concentrations of MSG and sucrose were based on prior studies (Chaudhari *et al.*, 1996; Stapleton *et al.*, 1999). The concentrations for the other four sweet substances were selected from the concentration–response gradients established in experiment 1 and were based on the following criteria: one concentration was selected from those that showed minimum suppression (60–80% of water), one concentration showing moderate suppression (40–60%) and one concentration showing strong suppression (<40%). In addition, rats were tested with 25 mM *N*-methyl-D-aspartate (NMDA) (pH adjusted to 6.7–7.0). Since an aversion to NMDA does not generalize to MSG (Stapleton *et al.*, 1999), NMDA served as a negative control to ascertain whether or not LiCl rats were simply avoiding any unknown taste stimulus ('dirty water effect') (Spector and Grill, 1988). All rats were also tested to assess whether the CTA to each CS generalized to 100 mM sucrose, with the exception of the rats tested for generalization between sucrose and MSG. Instead, these rats were presented with 100 mM NaCl to determine if conditioning to Na^+ taste might be occurring. On conditioning and test days, 30 μM amiloride was added to all taste stimuli. All seven solutions were presented twice during the test session. The order of stimulus presentation to each rat was randomized using a latin square. Stimuli were separated by 1–3 water trials.

Results and discussion

Lick rate data were normalized and scores for 'zero' concentrations were computed as described for experiment 1. Then the data for all rats tested with each sweet substance were examined with three-way ANOVA procedures for mixed designs treating the CS (2) and the US (2) variables as between subject and the taste stimuli (8) variable as repeated measures. In general, these ANOVAs indicated that the degree of aversion to MSG and to each sweet-eliciting substance was comparable and bidirectional for the concentrations tested. Thus, we are reporting the results of only the two-way ANOVAs performed on the data for each CS condition. The main effects for US and taste stimuli, and the interaction between these variables were significant in all of these ANOVAs [all interactions had an $F(7,98) = 9.47$ or greater, $P < 0.001$]. Consequently, to simplify the data presentation, only the corresponding simple effects tests are reported (Howell, 1997). All simple effects tests reported below are for $F(1,14)$ degrees of freedom.

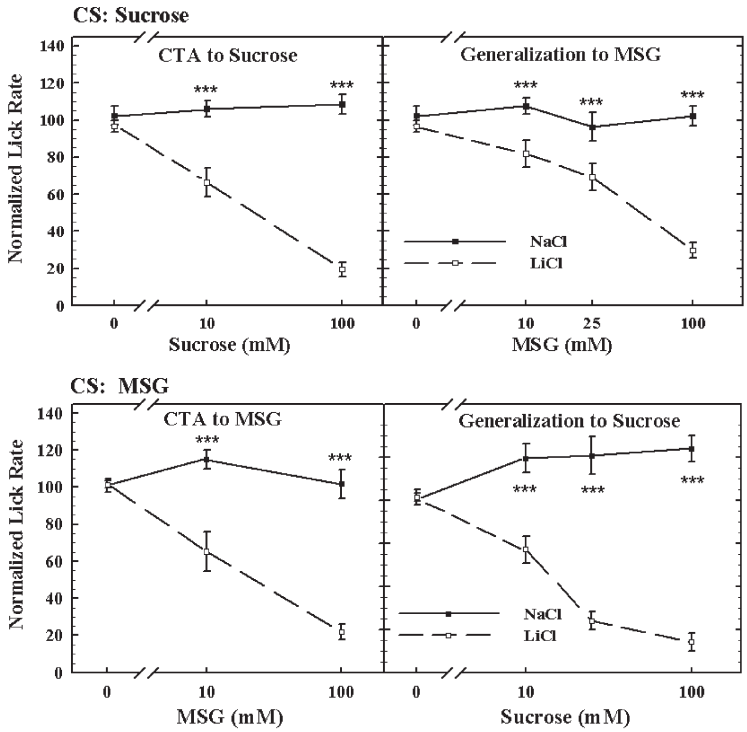

Figure 2 Rats conditioned to avoid 100 mM sucrose (top, left panel) also avoided MSG during generalization testing (top, right panel). Conversely, rats conditioned to avoid 100 mM MSG (lower, left panel), generalized the avoidance to sucrose (lower, right panel). Data are presented in the same format as in Figure 1. ***$P < 0.001$.

Sucrose

The groups testing the generalization of CTA between sucrose and MSG were important for establishing a baseline against which the rest of the substances could be compared (see Figure 2). Of the animals conditioned with sucrose as the CS, the LiCl rats significantly reduced their drinking of 10 and 100 mM sucrose (CS) and of all three concentrations of MSG compared to the NaCl animals (all Ps < 0.001). Similarly, of the animals conditioned with MSG as the CS, the LiCl rats significantly reduced their drinking of 10 and 100 mM MSG and of 10, 25 and 100 mM sucrose compared to NaCl animals (all Ps < 0.001). A relatively small but significant ($P < 0.05$) reduction of lick rates for 100 mM NaCl was seen in LiCl rats conditioned to avoid MSG but not in LiCl rats conditioned to avoid sucrose (Table 2).

Glucose

In general, the rats showed good generalization of CTA between MSG and glucose (see Figure 3). Simple effects tests of the data for animals conditioned to avoid glucose as the CS showed that LiCl rats significantly avoided 150 mM glucose compared to control rats ($P < 0.001$). In addition, the LiCl rats significantly avoided 100 mM sucrose as well as 25 and 100 mM MSG (all $Ps < 0.001$). Simple effects tests of the data for animals conditioned to avoid MSG indicated that the LiCl animals significantly reduced their drinking to 10 and 100 mM MSG and to 100 mM sucrose (all Ps < 0.001). These animals also significantly reduced lick rates for 50 ($P < 0.001$) and 150 mM glucose ($P < 0.001$). Neither CTA altered intake of the NMDA solutions (Table 2).

Maltose

The groups testing maltose and MSG tended to show weaker generalization of CTA between these two substances (see Figure 4). The data for the LiCl rats conditioned with maltose showed strong conditioning to maltose at both 10 mM ($P < 0.001$) and 100 mM ($P < 0.001$) as well as generalization to 100 mM sucrose ($P < 0.001$). However, these rats decreased MSG intake only at the 100 mM concentration ($P < 0.05$). The LiCl rats conditioned with MSG avoided 10 mM ($P < 0.05$) and 100 mM MSG ($P < 0.001$) and sucrose solutions ($P < 0.001$). They also reduced their intake of maltose significantly at 25 and 100 mM maltose (both Ps < 0.05). Conditioning to neither CS significantly affected NMDA intake (Table 2).

Saccharin

Saccharin showed strong bidirectional generalization of CTA with MSG (see Figure 5). The data analysis of the groups for which saccharin was the CS revealed an aversion to saccharin detectable at 0.625 and 1.25 mM saccharin (Ps < 0.001) and to MSG at 25 and 100 mM (both Ps < 0.001). As expected, the aversion to saccharin also generalized to sucrose ($P < 0.001$) but not to NMDA. For the animals

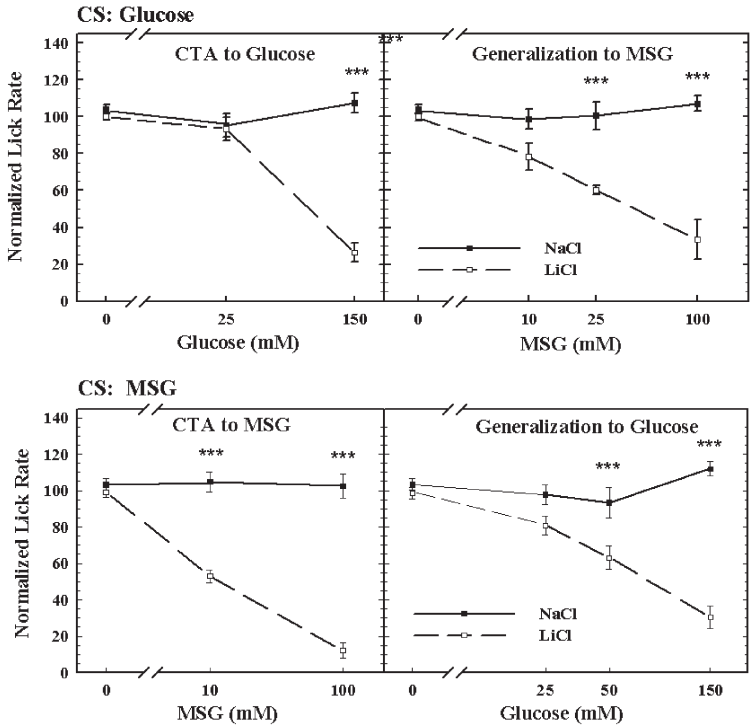

Figure 3 Rats conditioned to avoid 150 mM glucose (top, left panel) also avoided MSG during generalization testing (top, right panel). Conversely, rats conditioned to avoid 100 mM MSG (lower, left panel), generalized the avoidance to glucose (lower, right panel). Data are presented in the same format as in Figure 1. ***$P < 0.001$.

Table 2 Mean (SEM) normalized lick rates for control stimuli during generalization tests of experiment 2

Conditioned stimulus (CS)	Control stimulus					
	25 mM NMDA		100 mM sucrose		100 mM NaCl	
	Control[a]	Experimental[b]	Control	Experimental	Control	Experimental
150 mM glucose	98.81 (8.08)	91.75 (5.42)	104.72 (8.01)	23.41 (5.06)***		
100 mM maltose	96.79 (6.47)	101.12 (6.60)	96.48 (5.89)	52.98 (4.21)***		
1.25 mM saccharin	96.95 (5.73)	106.40 (4.17)	95.02 (9.22)	25.36 (4.32)***		
0.05 mM SC-45647	109.90 (5.92)	94.56 (7.17)	109.23 (7.36)	16.03 (3.20)***		
100 mM MSG[c]	99.99 (4.98)	95.47 (5.13)	110.41 (6.13)	31.72 (7.13)***		
100 mM sucrose	95.00 (4.79)	98.78 (4.55)			89.02 (7.63)	82.59 (9.94)
100 mM MSG[d]	88.83 (6.59)	89.29 (3.77)			101.06 (5.19)	82.46 (6.49)*

*$P < 0.05$; ***$P < 0.001$ (relative to their respective control groups).
[a]Control: each animal was conditioned with an injection of NaCl.
[b]Experimental: each animal was conditioned with an injection of LiCl.
[c]Normalized lick rates are averaged across all MSG groups in experiment 2 except those tested for generalization to sucrose.
[d]Normalized lick rates for the MSG rats tested for generalization to sucrose.

conditioned with MSG as the CS, the LiCl rats showed a similar pattern of generalization of their aversion. Relative to control rats, LiCl rats avoided MSG at 10 mM ($P < 0.01$) and 100 mM ($P < 0.001$). They also avoided 0.625 mM ($P < 0.01$) and 1.25 mM ($P < 0.001$) saccharin and the 100 mM sucrose ($P < 0.001$) but they did not avoid NMDA (Table 2).

SC-45647

SC-45647 is a potent artificial sweetener that also showed strong generalization of CTA with MSG (see Figure 6). When SC-45647 was the CS, the LiCl-conditioned animals clearly avoided both concentrations of SC-45647 compared to NaCl-conditioned rats, (Ps < 0.001). The LiCl animals

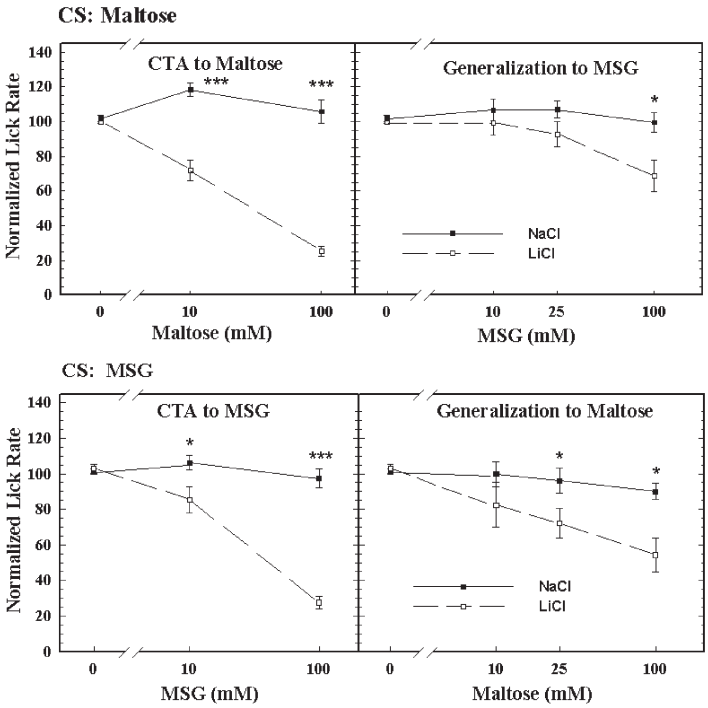

Figure 4 Rats conditioned to avoid 100 mM maltose (top, left panel) also avoided MSG during generalization testing (top, right panel). Conversely, rats conditioned to avoid 100 mM MSG (lower, left panel), generalized the avoidance to maltose (lower, right panel). Data are presented in the same format as in Figure 1. *$P < 0.05$; ***$P < 0.001$.

also showed an aversion to all three concentrations of MSG and to sucrose (all Ps < 0.001). When MSG was the CS, the LiCl animals, compared to NaCl animals, significantly decreased their lick rates for 100 mM MSG ($P < 0.001$) but not for 10 mM MSG. The LiCl animals also decreased their lick rates for 0.02 and 0.05 mM SC-45647 and for 100 mM sucrose (all Ps < 0.001) relative to control animals. Neither CTA affected drinking of NMDA (Table 2).

Comparisons between sweet-eliciting stimuli

To determine whether there were any differences in the degree of generalization between MSG and each of the five natural sugars and artificial sweeteners, separate analyses compared the magnitude of aversion for each CS and the magnitude of generalization to the test substance. First, the strengths of CTAs for each of the five substances were compared by examining the normalized lick rates for the highest concentration of each CS. No group differences were detected for rats conditioned with NaCl. Moreover, the reduction in drinking observed for rats conditioned with LiCl was similar for the five sweet-eliciting substances, suggesting that all of these rats showed comparable levels of aversion to their respective CS. All groups of rats conditioned with MSG as the CS also showed the same degree of aversion for 100 mM MSG.

Somewhat different results were revealed by the analyses performed on the normalized lick rates for the highest concentration of the taste stimuli used to test generalization (see Figure 7). No group differences in lick rates were found for any of the groups of rats conditioned with NaCl, whether the CS was MSG or one of the sweet-eliciting substances. However, there was a significant group difference detected in lick rates of LiCl rats with a CTA for one of the five sweet-eliciting substances [$F(4,35) = 7.28$, $P < 0.001$]. A Newman–Keuls test ($P < 0.01$) indicated that the rats conditioned to avoid maltose did not avoid MSG as much as the other groups of rats. A similar significant group difference was found for the LiCl rats conditioned to avoid MSG [$F(4,35) = 4.41$, $P < 0.01$]. The aversion to MSG did not generalize to maltose as much as it did to the rest of the sweet substances (Newman–Keuls test, $P < 0.025$). These results indicated that there was strong bidirectional generalization of CTA between MSG and four (sucrose, glucose, saccharin, SC-45647) of the sweet-eliciting substances and that, although there was also bidirectional generalization of CTA between MSG and maltose, this effect was significantly weaker than observed for the other four substances. Finally, the generalization of CTA to sucrose was not equivalent for all sweet substances [$F(3,28) = 16.04$, $P < 0.001$]. Newman–Keuls tests ($P < 0.01$) showed that, in comparison to other sweet substances, the aversion to maltose did not generalize as strongly to sucrose (Table 2).

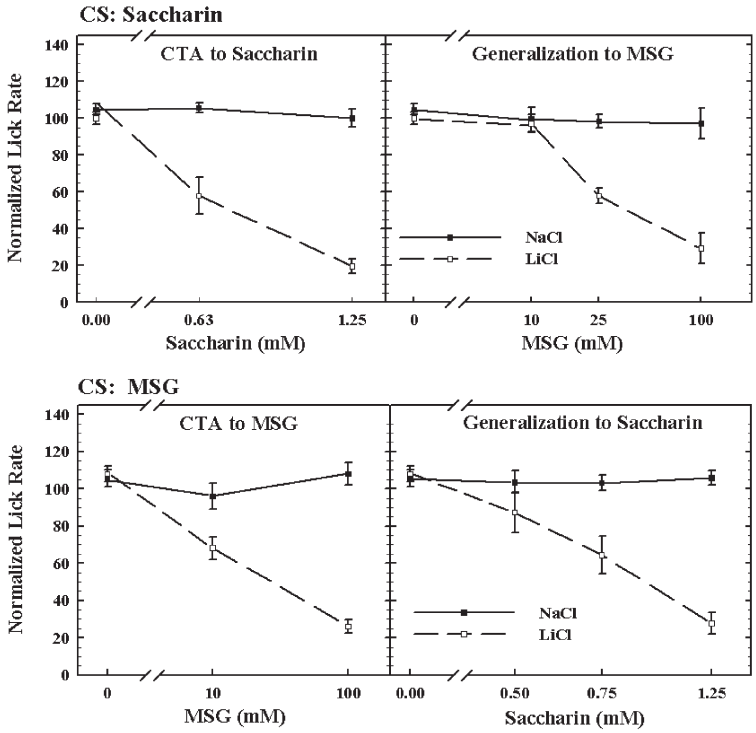

Figure 5 Rats conditioned to avoid 1.25 mM saccharin (top, left panel) also avoided MSG during generalization testing (top, right panel). Conversely, rats conditioned to avoid 100 mM MSG (lower, left panel), generalized the avoidance to saccharin (lower, right panel). Data are presented in the same format as in Figure 1. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

In summary, all of the groups conditioned with LiCl showed strong and comparable reductions in ingestion of their respective CS stimuli. In each case, these aversions generalized to the corresponding test stimulus. That is, those animals conditioned to avoid MSG also showed an aversion for each of the five natural sugars and artificial sweeteners, and conversely, those animals conditioned to avoid a substance that presumably elicits a sweet sensation in rats also avoided MSG. The magnitude of the generalization between maltose and MSG, and between maltose and sucrose, however, were less than for the other stimuli, even though the LiCl animals conditioned to avoid maltose showed the same level of CTA to maltose as the rest of the LiCl animals to their respective CS. All animals with a CTA also avoided 100 mM sucrose when used as a test stimulus. However, they did not avoid 25 mM NMDA. In the case of the LiCl animals in the sucrose experiment, only the MSG conditioned LiCl rats showed a mild aversion to 100 mM NaCl.

General discussion

One of the strengths of taste aversion paradigms is that once the subject learns the aversion, the subject will generalize that aversion to avoid any substance that elicits a similar taste. The greater the similarity between the tastes of the CS and the test substance, the more the subject avoids the test substance. Even though MSG is believed to elicit a unique umami taste in humans (Yamaguchi, 1967; Chaudhari *et al.*, 1996), early CTA experiments with rats reported that a taste aversion generalizes between sucrose and MSG when amiloride is present to reduce the taste of Na^+ (Yamamoto *et al.*, 1991; Chaudhari *et al.*, 1996; Stapleton *et al.*, 1999). Recently it was reported that rats have difficulty discriminating between sucrose and MSG when the taste of Na^+ was controlled by either adding amiloride to all solutions, adding equimolar concentrations of NaCl to sucrose, or both (Stapleton *et al.*, 2002). Collectively, these studies suggested that in rats the taste elicited by MSG mixed with amiloride is quite similar to sucrose, a substance which elicits a sweet taste in humans and is preferred by rats. The breadth of this 'sweet' component is much more clearly understood by the results of the present study. A CTA to MSG, mixed with amiloride, generalized universally to all five sweet substances tested in these experiments. Just as importantly, a CTA to each of the five substances tested in this study, whether it was a natural sugar or an artificial sweetener, generalized to MSG. However, this generalization was not because these rats were simply avoiding anything they detected since they did not avoid either 25 mM NMDA, a concentration that rats can detect easily (Stapleton *et al.*, 1999), or 100 mM NaCl in the sucrose experiment. Rather, these findings indicate that, at least to the rat, the 'sweet' component of MSG

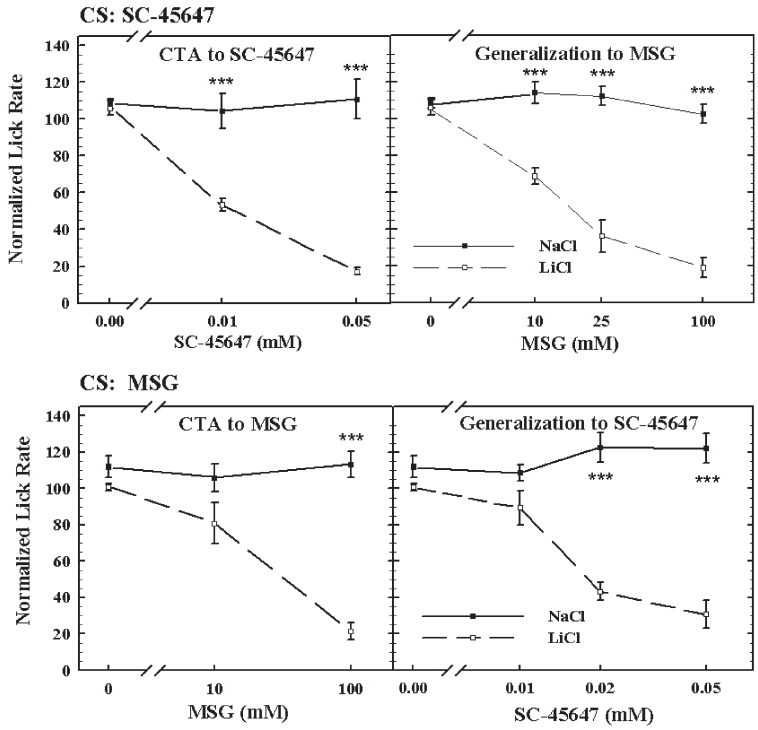

Figure 6 Rats conditioned to avoid 0.05 mM SC-45647 (top, left panel) also avoided MSG during generalization testing (top, right panel). Conversely, rats conditioned to avoid 100 mM MSG (lower, left panel), generalized the avoidance to SC-45647 (lower, right panel). Data are presented in the same format as in Figure 1. ***$P < 0.001$.

is broadly tuned and is most likely elicited by the glutamate anion.

The mechanism by which MSG elicits a taste sensation similar to these sweet substances, however, is unknown but could be at one or more levels in the afferent gustatory system, ranging from receptors and/or second-messenger transduction pathways found in taste receptor cells to central gustatory areas. For example, several researchers (Yamamoto *et al.*, 1991; Sako and Yamamoto, 1999; Ninomiya *et al.*, 2000) have suggested that glutamate activates receptors that evoke a sweet taste as well as receptors that evoke an umami taste. However, Li *et al.* (2002) reported that substances that elicit a sweet sensation activate T1R2+T1R3 receptors but had little or no effect on T1R1+T1R3 receptors activated by glutamate. There is also potential for interactions between second-messenger systems within taste receptor cells. MSG decreases cAMP within circumvallate and foliate taste receptor cells (Chaudhari *et al.*, 2000) and increases intracellular levels of cAMP and IP_3 in fungiform taste receptor cells (Nakashima and Ninomiya, 1998; Ninomiya *et al.*, 2000). It has also been well established that sweet stimuli can influence the same G protein-coupled second messenger pathways in taste receptor cells (Lindemann, 1996, 2001; Kinnamon, 2000). Natural sugars such as sucrose and presumably glucose and maltose, increase cAMP levels while artificial sweeteners such as saccharin and SC-45647 increase IP_3 levels in fungiform taste receptor cells (Bernhardt *et al.*, 1996; Nakashima and Ninomiya, 1998). At the brainstem level, within the solitary nucleus and the parabrachial nucleus of awake rats, neurons that responded best to sucrose, also responded strongly to MSG (Nishijo et al, 1991; Nakamura and Norgren, 1993). All of these data suggest that the afferent signals for MSG and stimuli perceived as sweet to humans may interact early in the afferent gustatory pathway. Although the behavioral data presented in this study cannot locate the point(s) of interaction between these stimuli, they clearly imply convergence in afferent signaling. Even though the sweet substances used in these experiments activate either cAMP (e.g., sucrose) or IP_3 (saccharin, SC-45647), the rats in these CTA experiments showed strong cross-generalization of CTA, and thus considerable perceptual similarities, between MSG mixed with amiloride and sweet-eliciting stimuli that activate either second messenger pathway. The breadth, strength, and uniformity of these generalizations, along with the data noted above, suggest substantial convergence between afferent signals for MSG and these sweet substances that may occur early in signal processing, e.g. within taste receptor cells or in peripheral fibers.

The results of these CTA experiments also suggest that there may be limits to the degree to which MSG mimics the taste of sweet-eliciting stimuli. Maltose was chosen as one of the stimuli because previous investigators reported that a CTA to sucrose generalizes only partially to maltose and

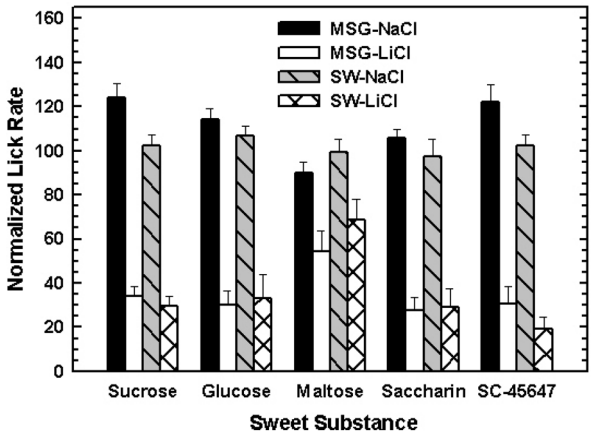

Figure 7 Mean (SEM) normalized lick rates (per format in Figure 1) for the highest concentration of the taste stimuli used to test generalization. The CS/US combinations during conditioning are indicated by: (1) MSG/NaCl = solid, black bars; (2) MSG/LiCl = solid, white bars; (3) sweet-eliciting substance/NaCl = diagonal lines, gray bars; and (4) sweet-eliciting substance/LiCl = cross-hatch lines, white bars. No group differences in lick rates were found for any of the groups of rats conditioned with NaCl, whether the CS was MSG or one of the sweet-eliciting substances. There was significant, strong bidirectional generalization of CTA between MSG and four (sucrose, glucose, saccharin, SC-45647) of the sweet-eliciting substances. Although there was also bi-directional generalization of CTA between MSG and maltose, this effect was significantly weaker than observed between MSG and the other four substances.

visa versa (Nissenbaum and Sclafani, 1987; Spector and Grill, 1988), a finding replicated in the current study. In fact, rats readily discriminate between the tastes of these two sugars (Spector *et al.*, 1997). Thus, while both substances may share some sensory characteristics, they are not perceptually similar and the afferent signaling for each of these two substances may be somewhat different. Interestingly, the CTA to MSG did not generalize to maltose as well as it did to other sweet stimuli and, conversely, the CTA to maltose did not generalize as strongly to MSG or to sucrose as the CTA for any of the other sweet stimuli, even though the concentrations that were tested were comparable and the degree of aversion for each CS was similar. These results along with those reported by other investigators suggest that the taste of maltose, although perceived as sweet by humans, is qualitatively different from the tastes of MSG with amiloride as well as other stimuli perceived as sweet by humans. Thus, maltose may activate at least some sweet-eliciting taste receptors, transduction mechanisms, or signaling in the afferent pathways that are not activated by glutamate or by sucrose, prospects also suggested recently by Chaudhari and Kinnamon (2001).

To summarize the main findings of this study, an aversion to MSG with amiloride added to reduce the taste of sodium, generalizes to sweet substances that activate either cAMP or IP_3 second messenger pathways in taste receptor cells. In turn, aversions to each of the sweet stimuli generalized in like manner to MSG with amiloride. The weak generalization between maltose and MSG and between maltose and sucrose suggested that maltose activates at least a subset of receptors or other afferent mechanisms not activated by the other substances. These results indicate that the taste elicited by the glutamate anion of MSG can mimic the taste of a number of natural sugars and artificial sweeteners, presumably through convergence of afferent signaling either in receptor cells or the afferent gustatory pathway.

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