Monosodium Glutamate but not Linoleic Acid Differentially Activates Gustatory Neurons in the Rat Geniculate Ganglion

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Abstract

To date, only one study has examined responses to monosodium glutamate (MSG) from gustatory neurons in the rat geniculate ganglion and none to free fatty acids. Accordingly, we recorded single-cell responses from geniculate ganglion gustatory neurons in anesthetized male rats to MSG and linoleic acid (LA), as well as to sucrose, NaCl, citric acid, and quinine hydrochloride. None of the 52 neurons responded to any LA concentration. In contrast, both narrowly tuned groups of gustatory neurons (sucrose specialists and NaCl specialists) responded to MSG, as did 2 of the broadly tuned groups (NaCl generalist$_I$ and acid generalists). NaCl-generalist$_{II}$ neurons responded only to the highest MSG concentration and only at low rates. No neuron type responded best to MSG; rather, responses to 0.1 M MSG were significantly less than those to NaCl for Na$^+$-sensitive neurons and to sucrose for sucrose specialists. Interestingly, most Na$^+$-sensitive neurons responded to 0.3 M MSG at levels comparable with those to 0.1 M NaCl, whereas sucrose specialists responded to 0.1 M MSG despite being unresponsive to NaCl. These results suggest that the stimulatory effect of MSG involves activation of sweet- or salt-sensitive receptors. We propose that glutamate underlies the MSG response of sucrose specialists, whereas Na$^+$-sensitive neurons respond to the sodium cation. For the latter neuron groups, the large glutamate anion may reduce the driving force for sodium through epithelial channels on taste cell membranes. The observed concentration-dependent responses are consistent with this idea, as are cross-adaptation studies using 0.1 M concentrations of MSG and NaCl in subsets of these Na$^+$-sensitive neurons.

Introduction

The mammalian gustatory system responds to a large number of chemical compounds that frequently are divided into a small number of perceptual categories, typically sweet, salty, sour, and bitter, with umami or fat taste occasionally included in the list of primary tastes. The latter has intuitive appeal because the archetypal stimuli for umami and fat taste are glutamate and fatty acids, which, in turn, represent crucial nutrients, protein and essential fatty acids. More importantly, glutamate and free fatty acids are reported to elicit sensations in humans and rats that are qualitatively different from the 4 classic primary tastes (Schiffman and Gill 1987; Hettinger et al. 1996; Lindemann et al. 2002; McCormack et al. 2006; Stratford et al. 2006a). Consistent with this notion, monosodium glutamate (MSG) and fat often are used as food additives to increase flavor and palatability.

It has been argued that umami taste is a composite of salty and sweet tastes. In fact, conditioned taste aversion (CTA) studies in rats and mice have shown that MSG cross-generalizes with a number of sweet stimuli when amiloride is added to the solutions to reduce the Na$^+$ taste component (Yamamoto et al. 1991; Chaudhari et al. 1996; Stapleton et al. 1999; Heyer et al. 2003). These studies have been taken as evidence that there is a sweet component to MSG taste; however, it should be noted that CTA studies indicate whether taste stimuli are similar, not whether they have perceptually unique taste qualities. Better resolution to analyze similarities and differences among a variety of taste stimuli may be achieved using taste discrimination methods, as was done by Heyer et al. (2004). In this study, rats were unable to discriminate sucrose from MSG when amiloride was added to the solutions, even though they easily discriminated glucose from both sucrose and MSG (with amiloride). Molecular studies have shown that the ligand-binding receptors for both umami and sweet are from the T1R family of G-protein–coupled receptors, although there are differences in the heterodimers that constitute the umami (T1R1 + 3) and sweet (T1R2 + 3) receptors (Nelson et al. 2001, 2002;

Li et al. 2002). Together, these studies suggest that MSG shares taste qualities with sucrose, likely due to the glutamate component, but behavioral studies to directly examine similarities and differences between the sodium component of MSG and salt taste remain to be done.

It frequently has been suggested that fat enhances taste stimuli. However, there are only small amounts of data to address the issue. A free fatty acid transporter is expressed in the gustatory epithelium (Fukuwatari et al. 1997), suggesting that the breakdown of fats may occur rapidly in the oral cavity and activate receptors therein. Consistent with this observation, Gilbertson has shown that delayed-rectifying potassium channels in isolated taste receptor cells are inhibited by fatty acids (Gilbertson et al. 1997; Gilbertson 1998). Several recent behavioral studies demonstrated that rats form a CTA to free fatty acids (McCormack et al. 2006; Stratford et al. 2006a). Interestingly, Stratford et al. (2006a) showed that bilateral transection of the rat chorda tympani nerve increases, but does not abolish, the detection threshold for linoleic acid (LA), suggesting that LA taste detection is, in part, dependent on the chorda tympani nerve.

This latter observation raises a critical question in determining how fat and MSG tastes are coded: do individual gustatory neurons in the periphery respond to MSG or to free fatty acids? We are unaware of any studies that have addressed this question in regard to free fatty acids. In contrast, a few in vivo studies have examined the responses of peripheral gustatory neurons in the rat to MSG (Sato et al. 1970; Boudreau et al. 1983; Yamamoto et al. 1991; Sako et al. 2000; Formaker et al. 2004). Although studies of higher order gustatory neurons suggest that umami taste responses are a composite of its sweet and salty taste qualities (Adachi and Aoyama 1991; Nishijo et al. 1991; Nakamura and Norgren 1993), significant processing of the neural input likely has occurred at these levels of the central nervous system that necessitate cautious interpretation of these results. To date, only one study has recorded responses to umami from the cell bodies of peripheral gustatory neurons located in the geniculate ganglion (Boudreau et al. 1983) and none has examined responses to LA. The advantages of the geniculate ganglion preparation for understanding gustatory coding have been firmly established by Boudreau's pioneering work (Boudreau and Alev 1973; Boudreau and White 1978; Boudreau et al. 1982 1983, 1985; Boudreau 1986, 1987). However, although the preparation has been used to test multiple taste stimuli, studies typically examined only one concentration of each stimulus. Accordingly, we examined single-cell responses of gustatory neurons in the geniculate ganglion, characterized on the basis of their responses to the 4 classic tastes, to 4 concentrations of MSG and 4 concentrations of LA. We also assessed the extent to which NaCl and MSG excite gustatory neurons through similar mechanisms by adapting the tongue to one of the 2 stimuli and then stimulating with the other.

Materials and methods

Animals and surgery

Adult male Sprague–Dawley rats (Charles River Laboratories; $n = 13$) weighing 287–575 g were housed individually in plastic cages in a temperature-controlled colony room on a 12:12 h light:dark cycle with lights on at 0700 h. All animals had free access to Purina Rat Chow (#5001) and deionized water (dH$_2$O). Rats were anesthetized with urethane (1.5 g/kg body weight) and, following a tracheotomy, were secured in a stereotaxic instrument with blunt ear bars. The tongue was gently extended and held in place by a suture attached to the ventral surface. The geniculate ganglion was exposed using a dorsal approach following procedures described previously (Lundy and Contreras 1999; Breza et al. 2006). Briefly, a midline incision was made on the occipital portion of the skull, and the skin and muscles were excised. A portion of the right cranium between bregma and lambda was removed, and the underlying neural tissue was aspirated to allow access to the temporal bone. The petrous portion of the temporal bone then was gradually planed away to expose the geniculate ganglion.

Extracellular single-cell electrophysiology was used to record activity from gustatory neurons in the geniculate ganglion. Epoxylite-insulated tungsten electrodes (2–6 MΩ; FHC, Bowdoinham, ME) were lowered into the ganglion using a stereotaxic micromanipulator (Siskiyou Design Instruments, Grants Pass, OR). Neural activity was differentially amplified (10 000×) with respect to an indifferent electrode attached to the skin overlying the cranium.

Stimulus delivery and stimulation protocols

Solutions were presented to the anterior portion of the tongue using a custom-built, computer-controlled fluid delivery system (Florida State University; R. Henderson), which allows stimuli to be presented at a constant flow rate of 50 μl/s, approximating the volume of fluid consumed by a rat licking from a drinking spout at a rate of 6–7 licks/s (Smith et al. 1992). The fluid delivery system's mixing port allows switching between taste stimuli or concentrations without compromising the flow rate. The computer program also controls a Peltier heat exchange device placed near the end of the stimulus outflow tube, which allows the temperature of the solutions to be held constant at 35 °C (±0.3 °C).

We tested each neuron's response to 15 s of stimulation with 1) the basic taste stimuli: 0.5 M sucrose, 0.1 M NaCl, 0.01 M citric acid, and 0.02 M quinine hydrochloride (QHCl); 2) MSG at 0.02, 0.04, 0.1, and 0.3 M; and 3) LA at 11, 22, 44, and 88 μM. LA concentrations were chosen based on previous studies from our laboratory and others (McCormack et al. 2006; Stratford et al. 2006a) that established the behavioral relevance of these concentrations. Due to its lipophilic nature, LA (99% pure; Sigma, St Louis, MO)

first was dissolved in 100% ethanol (EtOH) and then diluted to 88 μM LA and 5 mM EtOH. EtOH at this concentration does not affect behavioral taste responses (McCormack et al. 2006; Stratford et al. 2006a) and thus was used for this investigation. All other reagent grade chemicals were mixed in dH$_2$O.

The tongue was adapted to 35 °C dH$_2$O, and responses to the basic taste stimuli were evaluated. All neurons then were tested with ascending concentrations of LA and with ascending concentrations of MSG. To minimize the effect of stimulus sequence on the obtained results, the order of testing (first with LA and then MSG or first with MSG and then with LA) was randomized. Responses of all neurons to the basic taste stimuli were evaluated a second time at the conclusion of the experiment to verify recording stability. For 90–120 s before and after the presentation of each taste stimulus, dH$_2$O (for the basic taste stimuli and MSG) or 5 mM EtOH (for LA) flowed continuously over the tongue.

For a subset of the neurons ($N = 21$), we assessed the effect of cross-adaptation with equimolar concentrations (0.1 M) of NaCl and MSG. Neurons were stimulated with 0.1 M NaCl for 75 s to evaluate responses to prolonged stimulation. Following a 2-min dH$_2$O rinse, these neurons were then stimulated with 0.1 M NaCl for 60 s followed immediately by 15 s of 0.1 M MSG, maintaining a constant flow rate during the transition from NaCl to MSG. Seventeen of these 21 neurons also were stimulated with 0.1 M MSG for 75 s and, following a 2-min dH$_2$O rinse, then were stimulated with 0.1 M MSG for 60 s followed immediately by 15 s of 0.1 M NaCl.

Data and waveform analysis

Neuronal responses were monitored online with an oscilloscope and audiomonitor and digitized using commercially available computer hardware and software (Spike 2; Cambridge Electronic Design, Cambridge, UK). Digitized responses were stored for off-line analysis using Spike 2 software. Spike templates were formed from sampled data on the basis of amplitude and waveform. Spikes were characterized using measurements of 75 points uniformly distributed over the sinusoidal waveform. Individual spikes were included in a template only if >60% of the points matched the template and the amplitude differed by <10%. The raw traces from 5 individual gustatory neurons shown in Figure 1 illustrate typical signal-to-noise ratios.

Spontaneous firing rates for each neuron were calculated as the average number of spikes per second during the 5 s immediately prior to each stimulus. Responses to taste stimuli were calculated as the difference between the spontaneous firing rate immediately prior to stimulation and the average number of spikes per second during the 15 s of stimulation. The Poisson distribution was used to detect significant changes ($P < 0.05$) from spontaneous firing rate during stimulation with each taste stimulus.

Neurons were categorized based on their responses to the initial presentation of the 4 basic taste stimuli (0.5 M sucrose, 0.1 M NaCl, 0.01 M citric acid, and 0.02 M QHCl) and 0.1 M MSG by a hierarchal cluster analysis using Pearson's product-moment correlation coefficient and average-linking method between subjects (SPSS Inc., Chicago, IL).
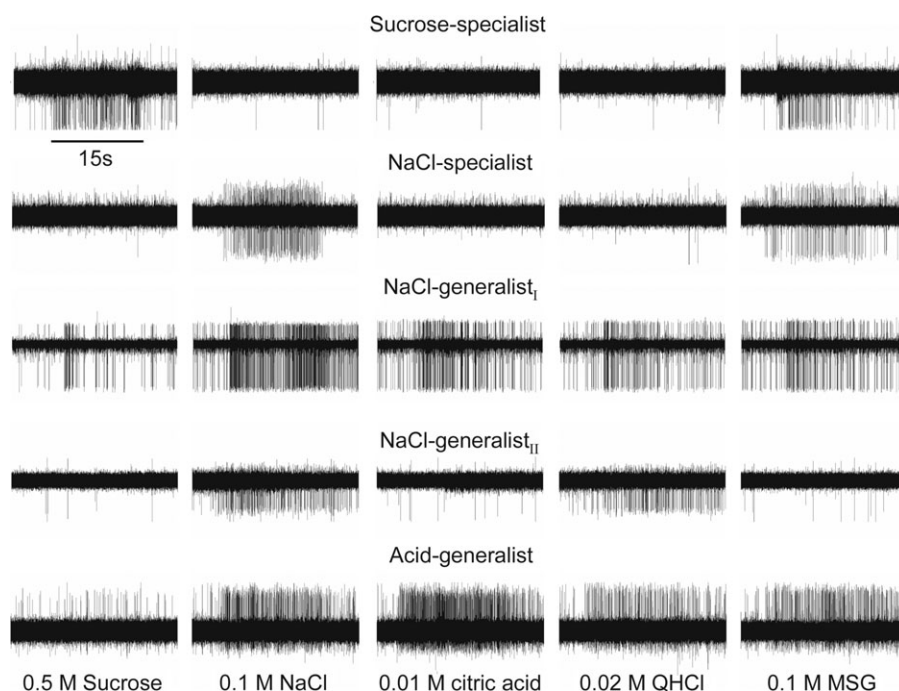
Figure 1 Raw electrophysiological traces from individual gustatory neurons in response to 0.5 M sucrose, 0.1 M NaCl, 0.01 M citric acid, 0.02 M QHCl, and 0.1 M MSG.

Responses to the 4 basic taste stimuli were used to determine the breadth of tuning (H) for each neuron, calculated as $H = -K \Sigma p_i \log p_i$, where K is a scaling constant (1.661 for 4 stimuli) and p_i is the proportion of the response to individual stimuli to which the neuron responded against the total responses to all the stimuli (Smith and Travers 1979). H values range from 0 to 1; 0 corresponds to neurons that responded to only one stimulus, and 1 corresponds to neurons that responded equally to all the stimuli. Thus, H values provide a quantitative measure of neurons as being narrowly or broadly tuned. Within each neuron group, paired T-tests were used to evaluate the breadth of tuning at the beginning and end of the protocol.

Further statistical analyses were conducted using appropriate analysis of variance (ANOVA; Statistica; StatSoft Inc., Tulsa, OK). Spontaneous firing rates prior to each group of stimuli (basic taste stimuli, MSG, and LA) were averaged for each neuron, and average baseline firing rates for each group of stimuli for each neuron group then were evaluated using 1-way ANOVAs. One-way repeated measures (RM) ANOVAs were used to evaluate the effect of stimulus (4 basic taste stimuli and 4 concentration of MSG) on firing rate (LA was not included as it was not an effective stimulus for gustatory neurons, as indicated by the Poisson distribution); 2-way RM ANOVAs were used to evaluate the responses to the 4 basic stimuli at the beginning and end of the recording (time × taste) and also to evaluate the effect of cross-adaptation on firing rate (condition × time). For the latter, we divided the 75-s stimulation period into fifteen 5-s bins and compared the number of spikes per second in each bin with the corresponding 5-s baseline spontaneous firing rate. Mauchly's test of sphericity was used to assess homogeneity of treatment differences variances, and, if sphericity was violated, Greenhouse–Geisser corrections were applied. Statistically significant main effects or interactions ($P < 0.05$) were further examined using Student Newman–Keuls tests. Data are presented as group means ± standard error.

Results

Neuron classification, spontaneous activity, and recording stability

We recorded responses from 52 gustatory neurons in the geniculate ganglion to 4 concentrations of MSG and to 4 concentrations of LA after characterizing neurons by their responses to lingual application with the 4 basic taste stimuli. Hierarchical cluster analysis grouped neurons on the basis of similarity of responses to the basic taste stimuli and 0.1 M MSG as shown in the dendrogram in Figure 2. Responses to 0.1 M MSG were included in the cluster analysis for comparison with those to 0.1 M NaCl; responses to LA were not included as the Poisson distribution indicated that LA was not an effective stimulus at any concentration tested.

Figure 2 Dendrogram showing the results of the hierarchical cluster analysis. Next to each neuron is the capital letter indicating the taste stimulus (S, 0.5 M sucrose; N, 0.1 NaCl; A, 0.01 M citric acid; Q, 0.02 M QHCl; M, 0.1 M MSG) that evoked the best response followed by small letters indicating taste stimuli that evoked a response ≥30% of the best response.

As in our previous investigation (Breza et al. 2006), cluster analysis revealed 5 groups of neurons that responded differently to taste stimuli (see Figure 1 for examples). Evaluation of breadth of tuning (Table 1) identified 2 groups of narrowly tuned specialist neurons (sucrose specialists and NaCl specialists) that responded only to sucrose or NaCl and 3 groups of broadly tuned generalist neurons that responded best to one of the basic taste stimuli and also to at least one

additional taste. Neurons classified as acid generalists responded best to citric acid. Similarly, neurons classified as NaCl generalists responded best to NaCl but separated into 2 distinct groups identified as NaCl generalists$_I$ and NaCl generalists$_{II}$. Figure 3 shows the response profiles of all 52 neurons grouped according to the cluster analysis and arranged within group by the stimulus evoking the best response.

Table 2 shows the average baseline spontaneous firing rates for each neuron group. It should be noted that 5 mM EtOH did not affect baseline firing rates. This finding was expected, as Lyall et al. (2005) reported that a 20% EtOH rinse solution did not increase the chorda tympani responses above baseline. More importantly, based on the Poisson distribution, 5 mM EtOH did not itself elicit excitatory responses. One-way ANOVA revealed a significant main effect of neuron type on spontaneous firing rate ($F_{4,47}$ = 4.97, P < 0.01), and post hoc tests showed that the spontaneous firing rates of NaCl generalists$_I$ and NaCl generalists$_{II}$ were significantly greater than those of sucrose specialists (P values < 0.05).

Within neuron groups, there were no differences in spontaneous firing rates throughout the protocol (Table 2) as

Table 1 Breadth of tuning, indicated by H-values, for each neuron group at the beginning and end of the protocol

Neuron type	Breadth of tuning	
	Beginning	End
Sucrose specialists	0.23 ± 0.03	0.18 ± 0.08
NaCl specialists	0.25 ± 0.04	0.28 ± 0.03
NaCl generalists$_I$	0.87 ± 0.03	0.87 ± 0.03
NaCl generalists$_{II}$	0.62 ± 0.04	0.63 ± 0.04
Acid generalists	0.78 ± 0.03	0.80 ± 0.03

Values shown are means ± standard error.

revealed by 1-way RM ANOVAs. Moreover, 2-way RM ANOVAs revealed no changes in response profiles or in response magnitude to any of the basic taste stimuli from the beginning to the end of the protocol (Table 3). Finally, evaluation of H values (Table 1) for each neuron group using paired T-tests revealed no changes in breadth of tuning from the beginning to the end of the protocol.

Responses to MSG and LA by neuron groups

All neurons responded with highest rates of firing to one of the 4 basic taste stimuli (Figure 3). No neuron responded to LA at any concentration. In contrast, many neurons responded to the 2 lowest concentrations of MSG, most were activated by 0.1 M MSG, and more than 90% increased firing to 0.3 M MSG (Table 4).

Sucrose-specialist neurons (n = 3)

One-way RM ANOVA revealed a significant main effect of taste ($F_{7,14}$ = 15.52, P < 0.001) on firing rates by sucrose specialists (Figure 4, top). Post hoc analyses showed that the response to sucrose was significantly greater than responses to all other basic taste stimuli and to 0.02, 0.04, and 0.1 M MSG (all P values <0.001) but was similar to that evoked by 0.3 M MSG. Thus, the order of stimulus effectiveness was sucrose = 0.3 M MSG > all other taste stimuli. Interestingly, 2 of the sucrose specialists responded to 0.1 M MSG (Table 4), whereas none responded to NaCl. As a group, however, the response to 0.1 M MSG was not different from that to 0.1 M NaCl, likely due to the small number of sucrose specialists.

NaCl-specialist neurons (n = 23)

One-way RM ANOVA with Greenhouse–Geisser correction revealed a significant main effect of taste ($F_{1.3,28.9}$ = 38.39,
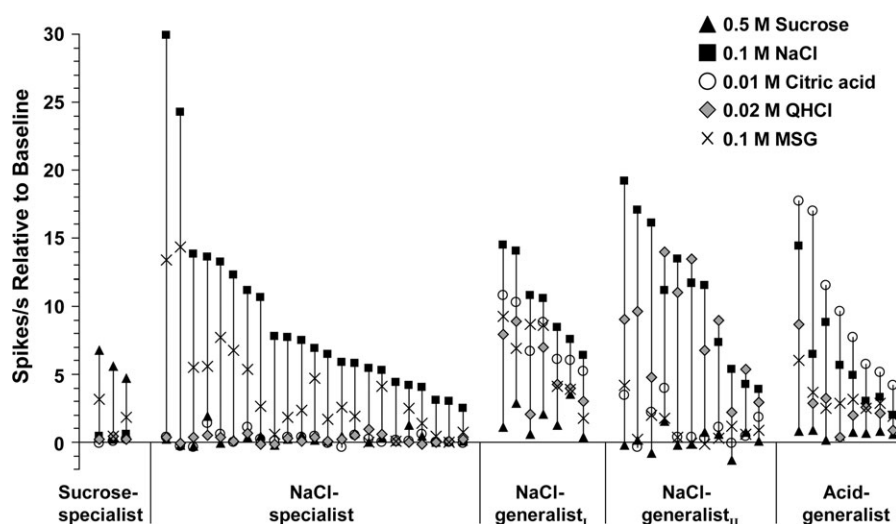

Figure 3 Responses by each neuron to 0.5 M sucrose, 0.1 M NaCl, 0.01 M citric acid, 0.02 M QHCl, and 0.1 M MSG. Within each group, neurons were arranged by response (spikes per second relative to baseline) to the best stimulus in descending order from left to right.

$P < 0.001$) on firing rates by NaCl specialists (Figure 4, bottom). Post hoc analyses showed that the response to NaCl was significantly greater than responses to all other basic taste stimuli and to 0.02, 0.04, and 0.1 M MSG (all P values <0.001) but was similar to that evoked by 0.3 M MSG.

NaCl specialists tended to respond to MSG in a concentration-dependent manner, with firing rates to 0.02 M MSG = 0.04 < 0.1 < 0.3 M (P values < 0.01). Of these 23 neurons, 18 responded to 0.1 M MSG, albeit moderately, whereas 22 of 23 neurons responded to 0.3 M MSG (Table 4).

NaCl-generalist$_I$ neurons (n = 7)

One-way RM ANOVA revealed a significant main effect of taste ($F_{7,42} = 18.86$, $P < 0.001$) on firing rates by NaCl generalists$_I$ (Figure 5, top). The response to NaCl was significantly greater than responses to all other basic taste stimuli and to MSG at all concentrations (all P values <0.05). In regard to the basic taste stimuli, the response to citric acid was greater than that to QHCl, which was greater than that to sucrose (all P values <0.05).

All 7 neurons responded moderately to 0.1 M MSG (Table 4). Moreover, NaCl generalists$_I$ tended to respond to MSG in a concentration-dependent manner, with gradual increases in firing to increasing MSG concentration. Specifically, the

response to 0.02 M MSG was comparable with 0.04 M but significantly less than those to 0.1 and 0.3 M MSG (P values < 0.01). Similarly, the response to 0.04 M MSG was comparable with 0.1 M but significantly less than that to 0.3 M ($P < 0.01$), and the response to 0.1 M MSG was comparable with 0.3 M MSG.

The response to citric acid by NaCl-generalist$_I$ neurons was significantly greater than those to 0.02 and 0.04 M MSG (P values < 0.01) and was comparable with those evoked by 0.1 and 0.3 M MSG. The response to QHCl was significantly greater than that to 0.02 M MSG ($P < 0.01$), comparable with those evoked by 0.04 and 0.1 M MSG, and significantly less than that to 0.3 M ($P < 0.05$).

NaCl-generalist$_{II}$ neurons (n = 11)

One-way RM ANOVA with Greenhouse–Geisser correction revealed a significant main effect of taste ($F_{2.5,24.6} = 27.06$, $P < 0.001$) on firing rates by NaCl generalists$_{II}$ (Figure 5, bottom). The response evoked by NaCl was significantly greater than those to all other basic taste stimuli and to MSG at all concentrations (all P values <0.01). Responses to QHCl were significantly greater than all taste stimuli (all P values <0.001) except NaCl.

As shown in Figure 3, 7 of the 11 neurons responded best to NaCl and second best to QHCl, whereas 4 responded best to QHCl and second best to NaCl. Regardless of whether NaCl-generalist$_{II}$ neurons responded best to NaCl or QHCl, MSG at 0.02, 0.04, or 0.1 M had virtually no effect on NaCl-generalist$_{II}$ neurons (Table 4 and Figure 3); however, 8 of these 11 neurons did respond weakly to 0.3 M MSG (Table 4).

Acid-generalist neurons (n = 8)

One-way RM ANOVA with Greenhouse–Geisser correction revealed a significant main effect of taste ($F_{1.5,10.6} = 18.72$, $P < 0.001$) on firing rates by acid generalists (Figure 6). The response evoked by citric acid was significantly greater than those to the other basic taste stimuli and to MSG at all

Table 2 Baseline spontaneous firing rates (spikes/s) of each neuron group after adaptation to dH$_2$O (for basic taste stimuli and MSG) and to 5 mM ethanol (for LA)

| Neuron type	dH$_2$O (basic tastes)	dH$_2$O (MSG)	5 mM EtOH (LA)	dH$_2$O (basic tastes)
Sucrose specialists	0.22 ± 0.17	0.27 ± 0.09	0.37 ± 0.25	0.32 ± 0.22
NaCl specialists	0.43 ± 0.10	0.47 ± 0.13	0.42 ± 0.12	0.48 ± 0.15
NaCl generalists$_I$	1.25 ± 0.34	1.50 ± 0.31	1.42 ± 0.37	1.14 ± 0.23
NaCl generalists$_{II}$	1.58 ± 0.39	1.24 ± 0.31	1.36 ± 0.33	1.38 ± 0.31
Acid generalists	0.79 ± 0.10	1.06 ± 0.11	1.04 ± 0.12	1.08 ± 0.21

Values shown are means ± standard error.

Table 3 Responses (spikes/s) by each neuron group to each of the 4 basic taste stimuli at the beginning (1) and end (2) of the protocol

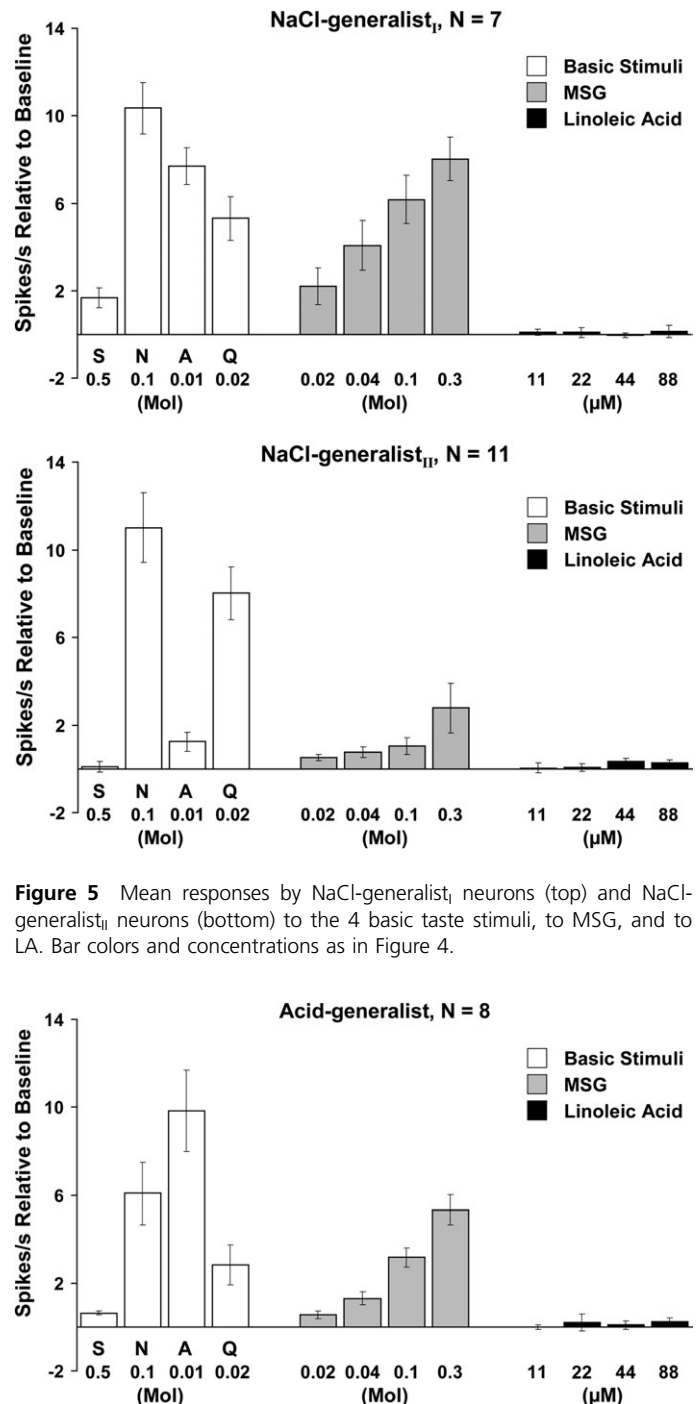|	Sucrose specialists	NaCl specialists	NaCl generalists$_I$	NaCl generalists$_{II}$	Acid generalists
Sucrose-1	5.7 ± 0.6	0.3 ± 0.1	1.7 ± 0.4	0.1 ± 0.2	0.6 ± 0.1
Sucrose-2	6.1 ± 0.9	0.4 ± 0.1	3.3 ± 0.8	0.6 ± 0.2	0.6 ± 0.1
NaCl-1	0.4 ± 0.1	9.1 ± 1.4	10.3 ± 1.2	11.0 ± 1.6	6.1 ± 1.4
NaCl-2	0.1 ± 0.0	9.8 ± 1.5	9.5 ± 1.4	11.0 ± 1.8	6.5 ± 1.1
Citric acid-1	0.1 ± 0.1	0.2 ± 0.1	7.7 ± 0.8	1.2 ± 0.4	9.8 ± 1.9
Citric acid-2	0.0 ± 0.1	0.7 ± 0.2	7.1 ± 1.2	1.2 ± 0.5	9.7 ± 1.7
QHCl-1	0.3 ± 0.1	0.2 ± 0.1	5.3 ± 1.0	8.0 ± 1.2	2.8 ± 0.9
QHCl-2	−0.2 ± 0.1	0.3 ± 0.1	5.5 ± 0.6	6.9 ± 1.4	3.5 ± 0.9

Values shown are means ± standard error.

Table 4 Percentage of neurons responding to MSG at 0.02, 0.04, 0.1, and 0.2 M as indicated by the Poisson distribution.

	MSG concentration (in M)			
	0.02	0.04	0.1	0.3
Total number of responsive neurons	31% (16/52)	48% (25/52)	73% (38/52)	92% (48/52)
Neuron type				
Sucrose specialists	0% (0/3)	0% (0/3)	67% (2/3)	100% (3/3)
NaCl specialists	35% (8/23)	52% (12/23)	78% (18/23)	96% (22/23)
NaCl generalists$_I$	57% (4/7)	86% (6/7)	100% (7/7)	100% (7/7)
NaCl generalists$_{II}$	18% (2/11)	18% (2/11)	27% (3/11)	73% (8/11)
Acid generalists	25% (2/8)	63% (5/8)	100% (8/8)	100% (8/8)
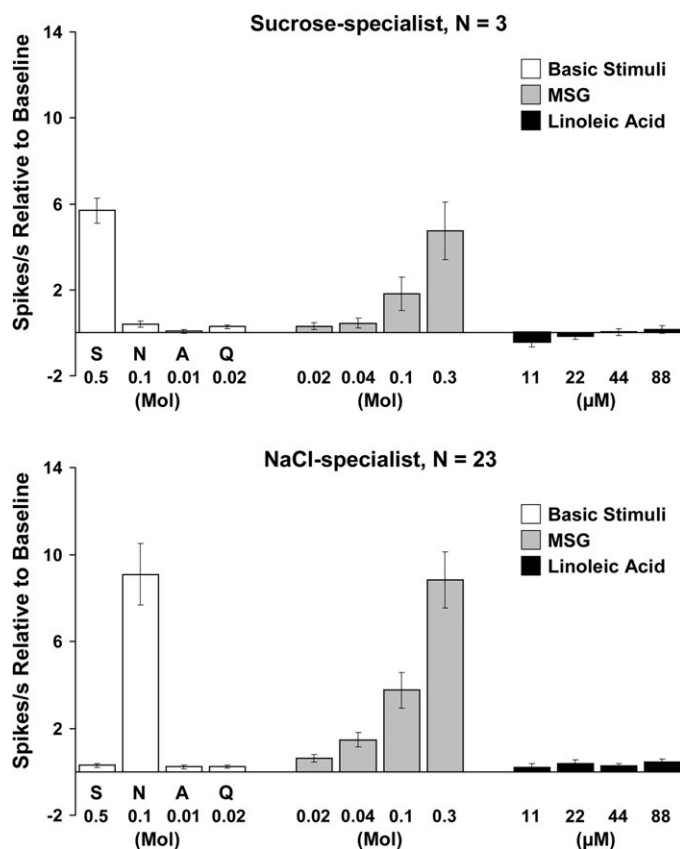
Figure 4 Mean responses (spikes per second relative to baseline) by sucrose-specialist neurons (top) and NaCl-specialist neurons (bottom) to the 4 basic taste stimuli (open bars), to MSG (gray bars; 0.02, 0.04, 0.1, and 0.3 M), and to LA (hatched bars; 11, 22, 44, and 88 μM).

Figure 5 Mean responses by NaCl-generalist$_I$ neurons (top) and NaCl-generalist$_{II}$ neurons (bottom) to the 4 basic taste stimuli, to MSG, and to LA. Bar colors and concentrations as in Figure 4.

Figure 6 Mean responses by acid-generalist neurons to the 4 basic taste stimuli, to MSG, and to LA. Bar colors and concentrations as in Figure 4.

concentrations (all *P* values <0.001). In regard to other basic taste stimuli, the response evoked by NaCl was significantly greater than that to QHCl, which was significantly greater than that to sucrose (all *P* values <0.05).

Acid generalists responded to MSG in a concentration-dependent manner, with 0.02 M = 0.04 M < 0.1 M < 0.3 M MSG (*P* values < 0.05). The response to NaCl was significantly greater than those to 0.02, 0.04, and 0.1 M MSG (all *P* values <0.05) but was comparable with that evoked by 0.3 M MSG. In contrast, the response to QHCl was significantly greater than those to 0.02 and 0.04 M MSG (*P* values < 0.05), comparable with 0.1 M MSG, and significantly less than that to 0.3 M MSG (*P* < 0.05).

Cross-adaptation

We used a cross-adaptation procedure involving prolonged stimulation with equimolar concentrations of NaCl and MSG to assess the contribution of the Na^+ cation to MSG responses in subsets of the groups of neurons that responded to NaCl (NaCl specialists, NaCl generalists$_I$, NaCl generalists$_{II}$, and acid generalists).

NaCl-specialist neurons

Figure 7A shows firing rate in 5-s bins during 75-s stimulation with 0.1 M NaCl (NaCl 75 s), and the effect of 60 s of NaCl adaptation on subsequent responses to 15-s stimulation with 0.1 M MSG (NaCl 60 s–MSG 15 s) by NaCl specialists ($N = 8$). NaCl responses tended to peak during the 15-s bin and declined steadily thereafter. A 2-way RM ANOVA revealed a significant main effect of time ($F_{15,105} = 12.74$, $P < 0.001$) on firing rates. As indicated by post hoc tests, firing rates reached levels significantly less than peak responses at the 35-s bin and at each bin thereafter (P values < 0.05) but remained significantly elevated from baseline throughout (P values < 0.05). There also was a significant interaction between condition and time ($F_{15,105} = 3.07$, $P < 0.001$), and post hoc tests showed that, after 60 s of adaptation to NaCl, MSG decreased the firing rate compared with the corresponding time in the NaCl

75-s condition ($P < 0.001$). Furthermore, the firing rate to MSG remained significantly less than that to NaCl for the remainder of the 5-s bins (P values < 0.05).

Figure 8A shows firing rate during 75-s stimulation with 0.1 M MSG (MSG 75 s) and with 60 s of MSG followed by 15-s stimulation with 0.1 M NaCl (MSG 60 s–NaCl 15 s) by NaCl specialists ($N = 8$). A 2-way RM ANOVA revealed a significant main effect of condition ($F_{1,7} = 11.30$, $P < 0.05$) and time ($F_{15,105} = 5.34$, $P < 0.001$) on firing rates. MSG responses tended to peak during the 20-s bin and, as indicated by post hoc tests of time, remained significantly elevated from baseline throughout (P values < 0.01). There also was a significant interaction between condition and time ($F_{15,105} = 5.47$, $P < 0.001$) and post hoc tests showed that, after 60 s of adaptation to MSG, NaCl increased the firing rate compared with the corresponding time in the MSG 75-s condition ($P < 0.001$). Furthermore, the firing rate to NaCl remained significantly greater than that to MSG for the remainder of the 5-s bins (P values < 0.01).

NaCl-generalist$_I$ neurons

Figure 7B shows firing rate during 75-s stimulation with NaCl and with 60 s of NaCl followed by 15 s of MSG by NaCl generalists$_I$ ($N = 4$). A 2-way RM ANOVA revealed
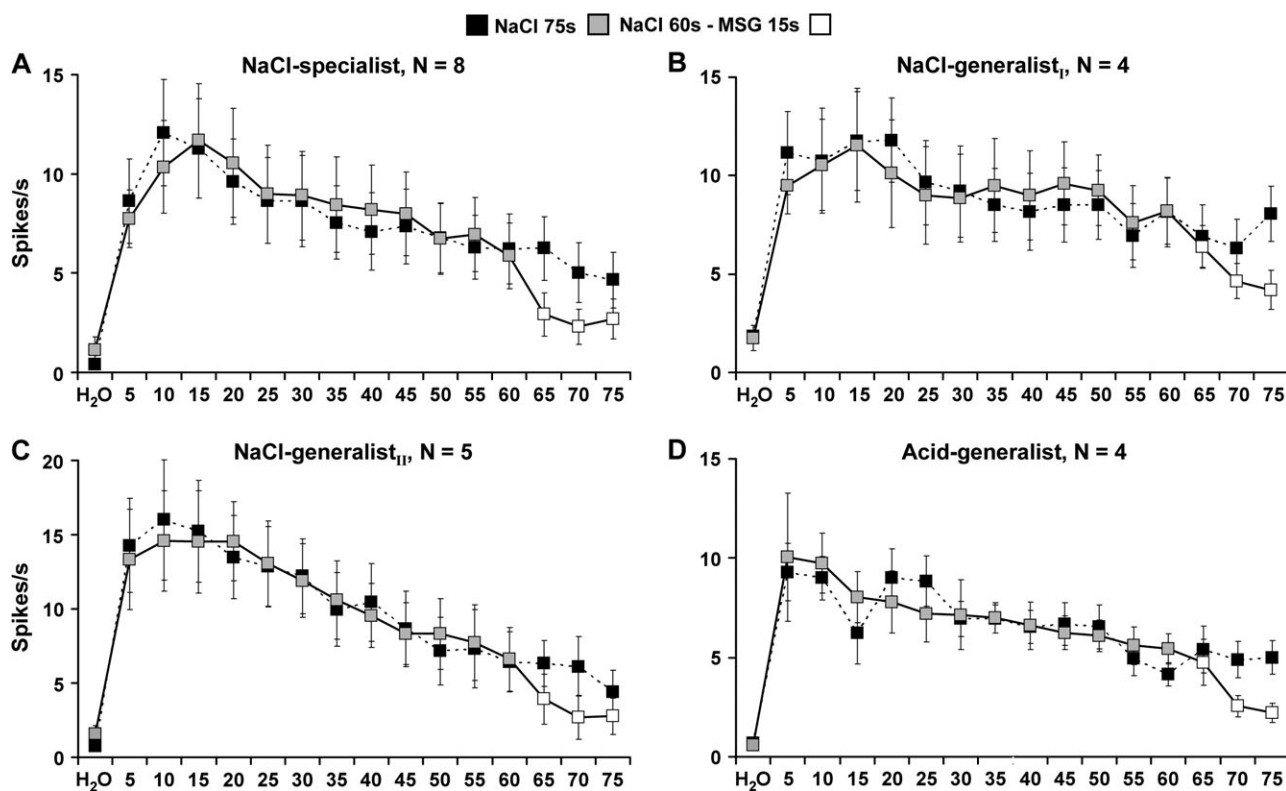

Figure 7 Mean responses (in 5-s bins) by **(A)** NaCl-specialist neurons; **(B)** NaCl-generalist$_I$ neurons; **(C)** NaCl-generalist$_{II}$ neurons; and **(D)** acid-generalist neurons to stimulation with 0.1 M NaCl for 75 s (black squares) and to cross-adaptation with 0.1 M NaCl for 60 s (gray squares) followed by 0.1 M MSG for 15 s (open squares).
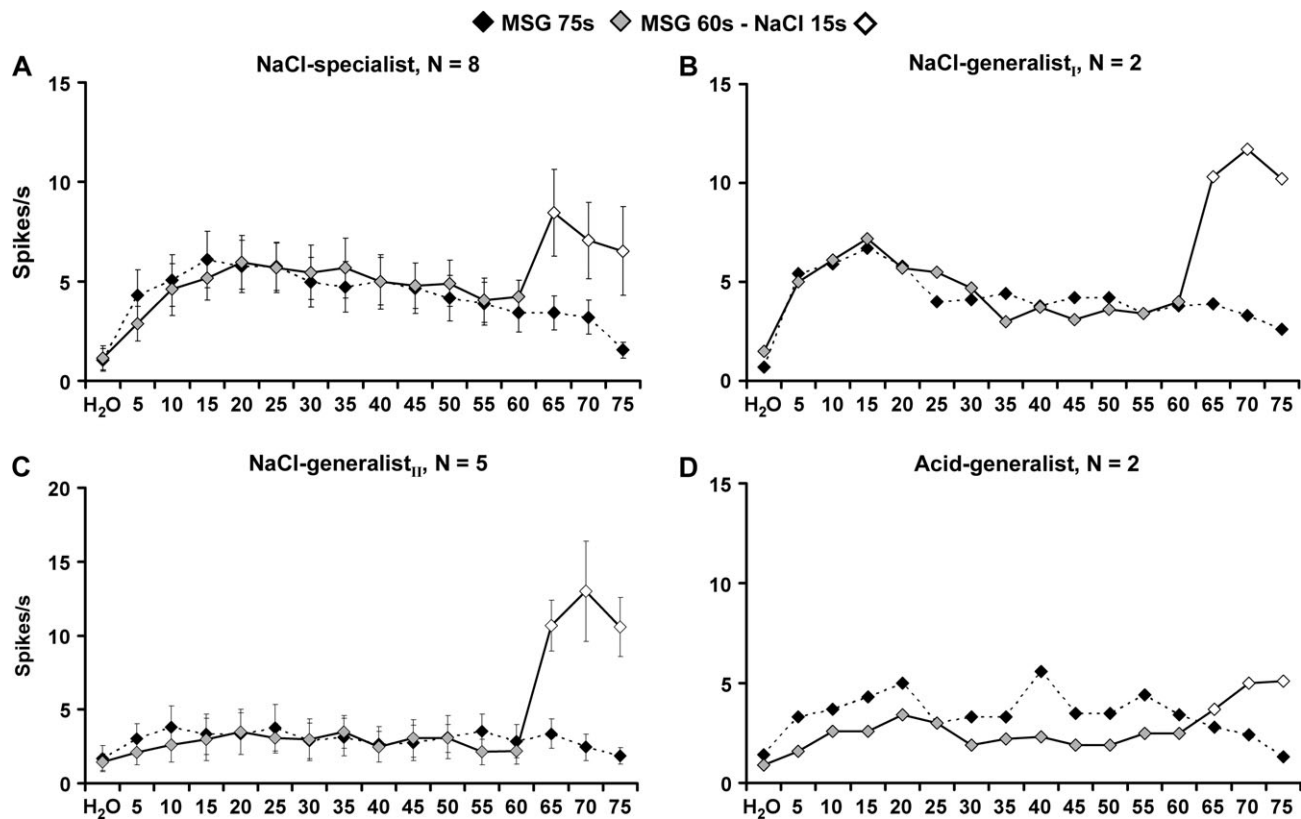
Figure 8 Mean responses (in 5-s bins) by **(A)** NaCl-specialist neurons; **(B)** NaCl-generalist$_I$ neurons; **(C)** NaCl-generalist$_{II}$ neurons; and **(D)** acid-generalist neurons to stimulation with 0.1 M MSG for 75 s (black diamonds) and to 0.1 M MSG for 60 s (gray diamonds) followed by 0.1 M NaCl for 15 s (open diamonds).

a significant main effect of time ($F_{15,45}$ = 10.77, $P < 0.001$). NaCl responses tended to peak during the 15-s bin and post hoc tests of time showed that responses did not change thereafter. There was no interaction between condition and time.

Figure 8B shows firing rate during 75-s stimulation with MSG and with 60 s of MSG followed by 15 s of NaCl by NaCl generalists$_I$ ($N = 2$). The data are shown only for purposes of comparison, as there were insufficient numbers of cells to make statistical comparisons.

NaCl-generalist$_{II}$ neurons

Figure 7C shows firing rate during 75-s stimulation with NaCl and with 60 s of NaCl followed by 15 s of MSG by NaCl generalists$_{II}$ ($N = 5$). NaCl responses tended to peak during the 10-s bin and declined steadily thereafter. A 2-way RM ANOVA revealed a significant main effect of time ($F_{15,60}$ = 9.64, $P < 0.001$), and post hoc tests indicated that firing rates reached levels significantly less than peak responses at the 45-s bin and at each bin thereafter (P values < 0.05). There also was a significant interaction between condition and time ($F_{15,60}$ = 2.41, $P < 0.01$). Post hoc tests showed that after 60 s of adaptation to NaCl, MSG decreased the firing rate abruptly compared with the corresponding time in the NaCl 75-s condition ($P < 0.05$). The firing rate to MSG remained significantly less than that to

NaCl for the first and second 5-s time bins (P values < 0.05) and, in fact, did not differ from baseline firing during these points.

Figure 8C shows firing rate during 75-s stimulation with 0.1 M MSG and with 60 s of MSG followed by 15-s stimulation with 0.1 M NaCl by NaCl generalists$_{II}$ ($N = 5$). A 2-way RM ANOVA revealed a significant main effect of time ($F_{15,60}$ = 6.33, $P < 0.001$); however, post hoc tests indicated that MSG responses remained largely unchanged from baseline throughout the 60 s of stimulation. There also was a significant interaction between condition and time ($F_{15,60}$ = 12.41, $P < 0.001$), and post hoc tests showed that, after 60 s of adaptation to MSG, NaCl increased the firing rate abruptly compared with the corresponding time in the MSG 75-s condition ($P < 0.001$). Furthermore, the firing rate to NaCl remained significantly greater than that to MSG for the remainder of the 5-s time bins (P values < 0.001).

Acid-generalist neurons

Figure 7D shows firing rate during 75-s NaCl stimulation and with 60 s of NaCl followed by MSG by acid generalists ($N = 4$). NaCl responses tended to peak during the 5-s bin and declined steadily thereafter. A 2-way RM ANOVA revealed a significant main effect of time ($F_{15,45}$ = 9.95, $P < 0.001$). Post hoc tests indicated that firing rates reached

levels significantly less than the peak responses at the 55-s bin and at each bin thereafter (P values < 0.01) but remained significantly elevated from baseline throughout (P values < 0.01). There also was a significant interaction between condition and time ($F_{15,45} = 1.99$, $P < 0.05$). Post hoc tests showed that, after 60 s of adaptation to NaCl, MSG decreased the firing rate compared with the corresponding time in the NaCl 75-s condition but only during the second and third 5-s time bins (P values < 0.05).

Figure 8D shows firing rate during 75-s stimulation with MSG and with 60 s of MSG followed by NaCl by acid generalists ($N = 2$). The data are shown only for purposes of comparison, as there were insufficient numbers of cells to make statistical comparisons.

Discussion

We recorded single-cell, electrophysiological activity from 52 gustatory neurons in the rat geniculate ganglion to lingual stimulation with the 4 classic taste stimuli and to 4 concentrations of MSG and 4 concentrations of LA. We observed interesting differences in the effectiveness of the 2 compounds to evoke responses, as well as in the pattern of responses by the different gustatory neuron types. Nonetheless, no neuron type displayed the highest rate of responding to LA or to MSG. The concentrations we used were based on previous studies, including our own (Gilbertson et al. 1997; Formaker et al. 2004; Heyer et al. 2004; Stratford et al. 2006a, 2006b); however, we cannot rule out the possibility that even higher concentrations of LA would have evoked a response in some neuron types, perhaps even a "best response," or that higher concentrations of MSG would have produced the highest rate of responding by some neuron types. Nonetheless, the present results clearly suggest that across these concentration ranges (which have been shown to elicit behavioral and/or electrophysiological responses) neither MSG nor LA is a "best" taste stimulus for geniculate ganglion neurons innervating taste receptors on the anterior portion of the tongue in rats.

We first classified neurons on the basis of their responses to the 4 basic taste stimuli and 0.1 M MSG. The neurons separated into 2 narrowly tuned groups (specialists) and 3 broadly tuned groups (generalists), similar in kind to those reported previously in studies of the rat geniculate ganglion (Lundy and Contreras 1999; Sollars and Hill 2005; Breza et al. 2006). As in our recent publication (Breza et al. 2006), we identified 3 neuron types that responded with the highest rates of firing to NaCl ("NaCl-best" neurons) when citric acid was used as the sour stimulus. Specifically, on the basis of the 4 basic taste stimuli, NaCl specialists responded exclusively to NaCl, whereas NaCl generalists$_I$ responded to NaCl, citric acid, and QHCl, and NaCl generalists$_{II}$ responded primarily to NaCl and to QHCl. Examination of the MSG responses supports the notion that these NaCl-best neurons function as 3 distinct neuron

groups. Moreover, the addition of MSG to our stimulus array for the cluster analysis further supports our contention (Breza et al. 2006) that neurons previously classified as QHCl generalists are in fact NaCl generalists$_{II}$ as these neurons, which responded to QHCl, were relatively unresponsive to both citric acid and MSG. Clearly, neurons that receive input from the foliate and circumvallate taste buds on the posterior tongue frequently respond best to bitter stimuli such as QHCl, as reported in studies recording from glossopharyngeal nerve fibers (Frank and Pfaffmann 1969; Frank 1991). Thus, NaCl-best geniculate ganglion cells, which receive input from receptors in the anterior tongue via the chorda tympani nerve, may have response profiles that are substantially different, both in terms of classic taste stimuli and to atypical stimuli like MSG and LA.

Linoleic acid

Based on previous work showing that transection of the chorda tympani nerve interfered with behavioral responses to LA (Stratford et al. 2006) and that free fatty acids modified the electrophysiological activity of isolated taste receptor cells (Gilbertson et al. 1997; Gilbertson 1998), we expected that gustatory neurons would respond to LA in vivo, though whether any neurons responded best to LA and whether the responsiveness would be limited to specific neuron types remained to be determined. Contrary to our expectations, however, none of the neuron types of the geniculate ganglion responded to any LA concentration. It seems clear, therefore, that LA is not a "best" stimulus; moreover, these surprising findings suggest LA—and perhaps other free fatty acids—may not be a taste stimulus at all. An alternative explanation for the results of behavioral studies suggesting that LA may be a taste stimulus comes from preliminary work from our laboratory (Stratford et al. 2005, 2006b). In these whole nerve recording studies, we showed that the chorda tympani was unresponsive to LA alone, but a mixture of LA and MSG elicited a whole nerve response that was larger than that to MSG alone. Thus, LA may modulate responses to other taste stimuli, and ongoing experiments are addressing this issue. An alternative possibility is that LA is an effective taste stimulus for receptive fields innervated by other taste nerves, though the full expression of LA's effect requires CT input, as well.

Monosodium glutamate

In general, MSG evoked responses from gustatory neurons: NaCl specialists, NaCl generalists$_I$ and acid generalists responded to MSG in a concentration-dependent manner; sucrose specialists responded at low rates to 0.1 M and at high rates to 0.3 M; and NaCl generalists$_{II}$ responded only at low rates and only to the highest concentration of MSG (0.3 M). However, no neuron type responded best (i.e., at highest rates) to MSG, suggesting that, like LA, MSG is not a best taste stimulus for geniculate ganglion neurons

innervating taste receptors on the anterior portion of the tongue in rats. Nonetheless, it is clear that MSG is an effective stimulus for gustatory neurons, with NaCl generalists$_{II}$ being the only exception.

Sato et al. (1970) showed that single fibers of the rat chorda tympani nerve that responded to MSG also responded to either NaCl or sucrose, suggesting that there are both sweet and salty components to MSG. Consistent with this idea, Yamamoto et al. (1988) showed that rats exhibited an aversion to the taste of MSG when conditioned to avoid the taste of NaCl. Moreover, rats were able to discriminate MSG from NaCl when amiloride was added to the solutions but then were not able to discriminate MSG from sucrose (Yamamoto et al. 1991). Taken together, these observations suggest that the sweet and salty components of MSG are coded by sucrose-sensitive and NaCl-sensitive neurons, respectively, and provide the context for the discussion of our findings.

Sucrose specialists

Sucrose specialists were unresponsive to NaCl but responded to MSG. This observation provides further support for the idea that MSG has both sweet and salty taste components and suggests that responses by sucrose specialists are attributable to the glutamate anion.

Twenty years ago, Schiffman and Gill (1987) proposed the existence of a glutamate receptor in taste receptor cells based on studies showing that lingual application of kainic acid, an analogue of glutamate, selectively depressed excitatory responses by neurons in the rat nucleus of the solitary tract to lingual glutamate stimulation. In the intervening years, understanding of sweet and umami receptors has increased dramatically. In no small part, these advances are due to studies that have elaborated the structure of ''sweet'' (T1R2 + T1R3) and ''umami'' (T1R1 + T1R3) receptors (Nelson et al. 2001, 2002; Li et al. 2002). These heterodimers appear to confer receptor specificity, as sweeteners such as sucrose did not activate cultured cells transfected with the T1R1 + T1R3 umami receptor, whereas amino acids, including glutamate, did not activate cells transfected with the T1R2 + T1R3 sweet receptor (Nelson et al. 2002).

But are there separate glutamate- and sucrose-sensitive neurons? Previous electrophysiological and calcium imaging studies have shown that individual taste receptor cells respond to multiple taste stimuli (Sato and Beidler 1997; Caicedo et al. 2002; Yoshida et al. 2006); however, none of those studies directly examined glutamate responses. On the surface, our observations that sucrose specialists in the rat geniculate ganglion responded to both sucrose and presumably to glutamate also would argue against distinct glutamate- and sucrose-sensitive neurons. Rather, individual taste receptor cells may respond to both stimuli by virtue of expressing receptors for both glutamate and sucrose. Although at present we cannot rule out this possibility as an explanation for our findings, alternative explanations focus on the innervation of the taste bud and rest on anatomical

evidence that a single geniculate ganglion cell innervates only one taste bud (Zaidi and Whitehead 2006). Thus, for example, cellular communication among taste receptor cells of multiple types within a taste bud (Yoshii K 2005) may underlie the ability of a single geniculate ganglion neuron to respond to multiple taste stimuli. Alternatively, nerve fibers may bifurcate at the base of the taste bud and synapse onto different receptor cells (e.g., one for sweet and one for umami). Clearly, much work is needed, but any of these possibilities, alone or in combination, may explain why sucrose-specialist neurons respond to sucrose and glutamate and, ultimately, why rats perceive sucrose and glutamate as similar taste qualities.

Na$^+$-sensitive Neurons

Na$^+$-sensitive neurons (NaCl specialists, NaCl generalists$_I$, NaCl generalists$_{II}$, and acid generalists), which overall were unresponsive to sucrose, responded to MSG, again supporting the idea that MSG has both sweet and salty taste components. More specifically, these observations suggest that Na$^+$-sensitive neurons responded to the Na$^+$ cation in MSG. However, the responses to stimulation with equimolar solutions of MSG and NaCl were not equivalent and, in fact, Na$^+$-sensitive neurons responded less robustly to 0.1 M MSG than to 0.1 M NaCl. A reasonable explanation for the differences in responses centers on the relative size of the anions, a phenomenon described by Beidler in the 1950s (see Beidler 1954) and commonly known as the ''anion effect.'' Subsequent investigations of the anion effect that focused on chorda tympani responses to a variety of sodium salts (Elliot and Simon 1990; Ye et al. 1991, 1993, 1994) showed that larger anions have reduced mobility through the paracellular shunt pathways. The resulting electropositive potentials on the outside of taste receptor cells hyperpolarizes the basolateral membrane, thereby decreasing the driving force for Na$^+$ into apical sodium channels and reducing the response to Na$^+$. Thus, it is likely that, in the present study, the large glutamate anion reduced the response to the Na$^+$ cation in MSG.

Consistent with work by Elliot and Simon (1990) demonstrating the anion effect in both the amiloride-sensitive and the amiloride-insensitive components of the chorda tympani response to salt, we found that both amiloride-sensitive NaCl specialists and amiloride-insensitive NaCl-generalist and acid-generalist neurons (Lundy and Contreras 1999) responded less to 0.1 M MSG than to 0.1 M NaCl. In fact, although 0.1 M MSG elicited responses from ∼80% of NaCl specialists and from 100% of NaCl generalists$_I$ and acid generalists, the responses were only ∼50 to 60% of those to 0.1 M NaCl. Responses to MSG attained levels comparable with those to 0.1 M NaCl only at 0.3 M MSG, which elicited responses from >95% of these neurons. NaCl-generalists$_{II}$ neurons were strikingly different from the other groups of Na$^+$-sensitive neurons, both in the percentage of neurons that responded at each concentration of MSG and in the

magnitude of the responses. Far fewer of these neurons (~30%) increased firing to 0.1 M MSG, and the response to 0.3 M MSG was substantially reduced compared with 0.1 M NaCl (~30%), despite the fact that more than 70% of these neurons responded to 0.3 M MSG. These observations may suggest that NaCl-generalists$_{II}$ neurons, which respond at high rates to 0.1 M NaCl and QHCl but not to the other basic taste stimuli, innervate taste receptor cells that use a different detection/transduction process. The Cl$^-$ anion is a likely candidate; however, Rehnburg et al. (1993) demonstrated that iontophoresis of Cl$^-$ to the anterior tongue elicited no response in the chorda tympani nerve. Thus, it seems more likely that the anion effect is simply more pronounced for NaCl-generalists$_{II}$ neurons.

Cross-adaptation

To further evaluate the contribution of the Na cation to MSG responses by Na$^+$-sensitive neurons, we conducted cross-adaptation tests with equimolar concentrations (0.1 M) of NaCl and MSG. Our working hypothesis was that if the responses were attributable to the Na$^+$ cation, neurons would not respond to MSG after prolonged stimulation with NaCl (and vice versa). Clearly, this was not the case: neurons increased firing to NaCl after prolonged stimulation with MSG and, interestingly, decreased firing to MSG after prolonged stimulation with NaCl.

On the surface, the MSG reduction of responses after prolonged NaCl stimulation might suggest that MSG activates Na$^+$-sensitive neurons via a non-Na$^+$ mechanism, perhaps the glutamate anion. Several observations argue against this explanation. First, even after adaptation to MSG, NaCl stimulation elicited increases in firing not appreciably different from peak responses to NaCl during prolonged stimulation or from those during 15-s stimulation. Similarly, even after adaptation to NaCl, the percent decrease in firing after MSG was similar to that observed when 60-s water rinses were interposed between 15-s stimulation with 0.1 M NaCl and with 0.1 M MSG. Moreover, in studies using hamster chorda tympani N fibers (NaCl best), Rehnburg et al. (1993) reported that, after 30 s adaptation to NaCl, sodium acetate decreased activity, whereas after 30 s adaptation to sodium acetate, NaCl increased activity. Finally, the responses to sodium acetate reported by Rehnburg et al. (1993) were similar in kind, but of less magnitude than those in the present investigation, and acetate is roughly half the size of glutamate. Thus, we suspect that our cross-adaptation studies also were influenced by the anion effect.

These cross-adaptation studies also reinforce our contention that 3 distinct NaCl-best neuron groups exist: the different neuron groups had unique patterns of response to prolonged stimulation with 0.1 M NaCl. For example, NaCl generalists$_I$ responded at fairly constant rates throughout NaCl stimulation (Figure 7B), and, after adaptation to NaCl, MSG did not affect firing rate. In contrast, NaCl

specialists, NaCl generalists$_{II}$, and acid generalists significantly decreased firing relative to peak responses during prolonged stimulation with NaCl; however, the firing rate declined most rapidly in NaCl generalists$_{II}$ (Figure 7C) and most slowly in acid generalists (Figure 7D). Despite these differences, after adaptation to NaCl, MSG decreased firing in NaCl specialists, NaCl generalists$_{II}$, and acid generalists. On the other hand, prolonged stimulation with 0.1 M MSG tended to elicit lower rates of firing by all groups of Na$^+$-sensitive neurons, as predicted from results when using brief stimulation. Firing rates appeared to change slightly or not at all during prolonged MSG stimulation; however, these results must be interpreted cautiously due to the smaller responses and, more importantly, to the small number of neurons in some groups. Nonetheless, after adaptation to MSG, NaCl appears to increase firing in all groups of Na$^+$-sensitive neurons.

Summary and conclusions

MSG and fat are commonly used as additives to increase the flavor and palatability of foods, but few in vivo studies have examined the responses of peripheral gustatory neurons in the rat to MSG and none have examined the responses to LA. We used the geniculate ganglion preparation to record responses to MSG and LA from rat gustatory neurons, which were classified based on responses to 0.5 M sucrose, 0.1 M NaCl, 0.01 M citric acid, 0.02 M QHCl, and 0.1 M MSG. Our expectations were that MSG or LA would be a "best" stimulus for a subset of taste neurons. However, none of the neuron groups showed the highest rate of responding to LA, and in fact, no neuron of any group responded to any concentration of LA. These results strongly suggest that LA is not an effective taste stimulus for anterior tongue receptors adapted to a water rinse. On the other hand, MSG evoked responses from all neuron groups, though none responded to MSG with the highest rate of firing. Thus, though there may be no MSG-best neurons in the rat geniculate ganglion innervating the anterior portion of the tongue, MSG certainly is an effective stimulus for these gustatory neurons. Moreover, based on responses by sucrose specialists (which responded to sucrose and MSG but not to NaCl) and by NaCl specialists (which responded to NaCl and MSG but not to sucrose), we conclude that the responses to MSG are attributable to its sweet (glutamate) and salty (Na$^+$) components, as previously shown in behavioral tests of rats. Clearly, sucrose specialists and NaCl specialists are ideally suited to explicate the mechanisms of MSG actions in the gustatory system and likely contribute substantially to coding the sweet and salty components of MSG. NaCl generalists$_I$, NaCl generalists$_{II}$, and acid generalists also responded to MSG—presumably to the Na$^+$ cation, as none responded to sucrose—though at present their roles in coding the salty component of MSG is less clear. For example, NaCl generalists$_{II}$ responded with high

rates of firing to 0.1 M NaCl but not to 0.1 M MSG, demonstrating that MSG does not invariably equal NaCl + sucrose.

In addition, results of these studies support our previous findings of 3 distinct groups of NaCl-best neurons. Compared with NaCl specialists and NaCl generalists$_I$, a smaller percentage of NaCl generalists$_{II}$ responded to MSG, and even at the highest concentration used, the response was, at best, slightly increased from baseline. Moreover, NaCl-best neurons differed in the latency to peak response to NaCl during prolonged stimulation, as well as in the temporal pattern of adaptation to prolonged NaCl stimulation. In the latter case, NaCl generalists$_I$ continued to fire at high rates throughout the 60- to 75-s stimulation protocol, whereas firing by NaCl specialists and NaCl generalists$_{II}$ declined dramatically. Interestingly, the magnitude of the MSG response was influenced by the anion effect in all NaCl-best neurons types, regardless of whether they were narrowly or broadly tuned. In fact, the anion effect also affected the MSG response by acid generalists, which respond to NaCl, though best to citric acid.

Taken together, these results strongly suggest that neither MSG nor LA is a best stimulus for geniculate ganglion neurons innervating taste receptors on the anterior portion of the tongue in rats. This additional information about differences and similarities in responses to ''atypical'' taste stimuli by groups of peripheral gustatory neurons may advance our understanding about the roles of these neuron types in gustatory coding.

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