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Temperature Modulates Taste Responsiveness and Stimulates Gustatory Neurons in the Rat Geniculate Ganglion

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Breza, Joseph M., Kathleen S. Curtis, and Robert J. Contreras. Temperature modulates taste responsiveness and stimulates gustatory neurons in the rat geniculate ganglion. *J Neurophysiol* 95: 674–685, 2006. First published November 2, 2005; doi:10.1152/jn.00793.2005. In humans, temperature influences taste intensity and quality perception, and thermal stimulation itself may elicit taste sensations. However, peripheral coding mechanisms of taste have generally been examined independently of the influence of temperature. In anesthetized rats, we characterized the single-cell responses of geniculate ganglion neurons to 0.5 M sucrose, 0.1 M NaCl, 0.01 M citric acid, and 0.02 M quinine hydrochloride at a steady, baseline temperature (adapted) of 10, 25, and 40°C; gradual cooling and warming (1°C/s change in water temperature >5 s) from an adapted tongue temperature of 25°C; gradual cooling from an adapted temperature of 40°C; and gradual warming from an adapted temperature of 10°C. Hierarchical cluster analysis of the taste responses at 25°C divided 50 neurons into two major categories of narrowly tuned (Sucrose-specialists, NaCl-specialists) and broadly tuned (NaCl-generalists_I, NaCl-generalists_{II}, Acid-generalists, and QHCl-generalists) groups. NaCl specialists were excited by cooling from 25 to 10°C and inhibited by warming from 10 to 25°C. Acid-generalists were excited by cooling from 40 to 25°C but not from 25 to 10°C. In general, the taste responses of broadly tuned neurons decreased systematically to all stimuli with decreasing adapted temperatures. The response selectivity of Sucrose-specialists for sucrose and NaCl-specialists for NaCl was unaffected by adapted temperature. However, Sucrose-specialists were unresponsive to sucrose at 10°C, whereas NaCl-specialists responded equally to NaCl at all adapted temperatures. In conclusion, we have shown that temperature modulates taste responsiveness and is itself a stimulus for activation in specific types of peripheral gustatory neurons.

INTRODUCTION

Subjective human experience, in conjunction with empirical evidence from psychophysical studies of humans, indicates that temperature influences taste perception and palatability (Cruz and Green 2000; Green 2002; Green and Frankmann 1988; Von Beskesy 1964). For example, Green and Frankmann (1988) showed that glucose and fructose were perceived as less sweet when the tongue temperature was cooled. Interestingly, in a recent study by Cruz and Green (2000), human subjects reported that warming the anterior portion of the tongue produced a sweet sensation, whereas cooling evoked a sour or salty taste sensation. Thus temperature also may influence gustatory processing independent of taste stimuli, as changes in tongue temperature alone appear to evoke taste perceptions.

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In a number of electrophysiological studies, temperature has been shown to influence gustatory sensory nerve activity in several animal species (Lundy and Contreras 1997; Nakamura and Kurihara 1991; Oakley 1985; Yamashita and Sato 1965; Yamashita et al. 1964). The chorda tympani nerve, which innervates fungiform taste buds on the anterior two-thirds of the tongue, responded maximally to the basic taste stimuli of sweet, salt, sour, and bitter when the tongue temperature was maintained between 30 and 35°C (Yamashita and Sato 1965; Yamashita et al. 1964). Recordings from the whole chorda tympani nerve, however, may be unable to detect the contribution of numerous individual fibers that have been categorized based on the taste stimulus evoking the best response (Erickson et al. 1965; Fishman 1957; Frank 1974; Frank et al. 1983, 1988; Hellekant et al. 1997a,b; Ninomiya et al. 1982; Sato et al. 1975; Yamashita et al. 1970). Better resolution can be obtained from electrophysiological recordings of single chorda tympani fibers. In fact, Ogawa et al. (1968) showed that HCl and quinine hydrochloride (QHCl) sensitivity in single fibers of the chorda tympani were positively correlated with cooling, whereas sucrose sensitivity was positively correlated with warming, in rats and hamsters, respectively. Furthermore, responses by individual chorda tympani fibers to either cooling or warming were similar to those evoked by taste stimuli (Ogawa et al. 1968).

Although these studies have furthered understanding of the relationship between taste and temperature, electrophysiological studies of both whole nerves and single fibers have the methodological disadvantage of recording from severed axons, which have limited viability. As an alternative, single-cell extracellular recordings can be obtained from cell bodies of chorda tympani nerve fibers, which are located in the geniculate ganglion. First described by Boudreau in 1973 (Boudreau and Alev 1973) and used in comparative studies throughout the early 1980s (Boudreau et al. 1978, 1982, 1983, 1985), the ganglion preparation allows stable recordings from individual neurons over hours, thereby providing detailed analyses of neuron response profiles as well as better resolution of the similarities and differences among multiple neuron types.

Lundy and Contreras (1999) examined the thermal sensitivity of gustatory neurons in the geniculate ganglion and found that geniculate ganglion neurons that responded best to acid also responded to a gradual (1°C/s) decrease in the temperature of the water bathing the tongue. However, only responses to cooling and from only one temperature (35°C) were examined. The responsiveness of geniculate ganglion neurons to the basic

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taste stimuli also was influenced by temperature; however, these preliminary data did not include all gustatory neuron types. To date, therefore, little is known about the thermal sensitivity of gustatory neuron types to cooling and warming or how the adapted temperature of the tongue influences taste responsiveness. The present study had two major goals: to characterize the electrophysiological responses of gustatory neurons in the geniculate ganglion to both cooling and warming and to determine the influence of temperature on the responsiveness of gustatory neurons to taste stimulation.

METHODS

Animals and surgery

Adult male Sprague-Dawley rats (Charles River Laboratories; $n = 14$) weighing 290–520 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 (No. 5001) and deionized water (dH_2O). Rats were anesthetized with urethan (1.5g/kg body wt) and after 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). 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. Epoxyite-insulated tungsten electrodes (2–7 M Ω ; FHC, Bowdoinham, ME) were lowered into the ganglion using a stereotaxic micromanipulator (Siskiyou Design Instruments; Grants Pass, OR). Neural activity was differentially amplified ($\times 10,000$) 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). The fluid delivery system allows stimuli to be presented at a constant flow rate of 50 $\mu\text{l/s}$, which approximates 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 computer program also controls a Peltier heat exchange device placed near the end of the stimulus outflow tube. The Peltier device allows the temperature of the solutions to be held constant ($\pm 0.3^\circ\text{C}$) or to be increased or decreased at a constant rate of 1°C/s . During the recording session, dH_2O flowed continuously over the tongue at 50 $\mu\text{l/s}$ for 60–90 s before and after the presentation of each taste stimulus.

We tested each neuron's response to 15 s of stimulation with the basic taste stimuli: 0.5M sucrose, 0.1M NaCl, 0.01M citric acid, and 0.02M quinine hydrochloride (QHCl) at a constant temperature (adapted) of 10, 25, and 40°C ; gradual cooling and warming from an adapted tongue temperature of 25°C ; and gradual cooling from an adapted temperature of 40°C and gradual warming from an adapted temperature of 10°C . Each thermal stimulus consisted of a change in the temperature of dH_2O bathing the receptors at a rate of 1°C/s over 15 s. We considered 25°C as the standard temperature because previous electrophysiological studies of taste, with a few exceptions, delivered taste solutions at room temperature.

Figure 1 shows the stimulation protocols used in the present study. Neurons were randomly assigned to be tested in *protocol A* or *protocol B* to minimize the effect of stimulus sequence on the obtained results. Both protocols began with the tongue adapted to 25°C dH_2O , followed by an evaluation of the neuron's responses to the basic taste stimuli. The temperature of dH_2O was increased to 40°C (*protocol A*, top) or decreased to 10°C (*protocol B*, bottom) by 1°C/s over a 15-s period. After a 4-min adaptation period to the new temperature, the neuron's responses to the basic taste stimuli were evaluated a second time. The 4-min adaptation period was chosen because it was sufficient time for each neuron to adapt to the new temperature and resume a steady, baseline rate of spontaneous activity. The temperature of dH_2O then was decreased to 25°C (*protocol A*) or increased to 25°C (*protocol B*) by 1°C/s over 15 s. After 4-min adaptation to this temperature, the neuron's responses to the basic taste stimuli were evaluated a third time. The temperature of the dH_2O then was decreased to 10°C (*protocol A*) or increased to 40°C (*protocol B*) by 1°C/s over 15 s. After 4-min adaptation, the neuron's responses to the four basic taste stimuli were evaluated a fourth time. Finally, the temperature of dH_2O was increased (*protocol A*, top) or decreased (*protocol B*, bottom) by 1°C/s over 15 s to the standard 25°C , and after 4-min adaptation, the neuron's responses to the basic taste stimuli were evaluated a fifth and final time. Thus the neuron's responses to the basic taste stimuli were evaluated three times at the standard 25°C temperature, once at 10°C , and once at 40°C .

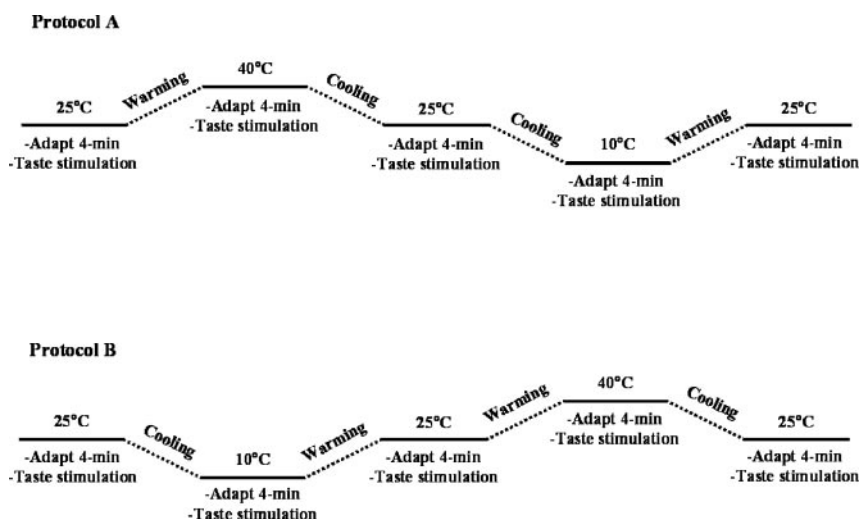


FIG. 1. Diagram illustrating the 2 protocols for delivering thermal and taste stimuli.

Data and waveform analysis

Neural responses were monitored on-line 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 five individual gustatory neurons shown in Fig. 4 illustrate typical signal-to-noise ratios.

Spontaneous firing rates for each neuron were calculated as the average number of spikes/s during the 15 s immediately prior to each stimulus. Responses to taste and thermal stimuli were calculated as the difference between the spontaneous firing rate immediately prior to stimulation, and the average number of spikes/s 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 and thermal stimulus. All data are presented as the mean \pm SE.

Neurons were categorized based on their responses to the initial presentation of the four basic taste stimuli at 25°C by a hierarchical cluster analysis using Pearson's product-moment correlation coefficient and average-linking method between subjects (SPSS; Chicago, IL).

Responses to the taste stimuli at 10, 25, and 40°C were used to determine the breadth of tuning (H) for each neuron, calculated as $H = -K \sum p_i \log p_i$, 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, where a value of 0 corresponds to neurons that responded to only one stimulus and a value of 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.

Further statistical analyses were conducted using appropriate ANOVA (Statistica; StatSoft, Tulsa, OK). Spontaneous firing rates prior to all stimuli at each adapted temperature were averaged for every neuron, and average baseline firing rates at each temperature then were evaluated using one-way ANOVAs for each neuron group. Within each neuron group, one-way repeated-measures (RM) ANOVAs were used to evaluate the effect of temperature on breadth of tuning; two-way RM ANOVAs were used to evaluate the effect of temperature on responses to the four taste stimuli (temperature \times taste) and the effect of thermal stimulation on firing rate (temperature \times time). We divided the 15-s stimulation period into three 5-s bins and compared the number of spikes/s in each bin with the corresponding baseline spontaneous firing rate. Statistically significant main effects or interactions ($P < 0.05$) were further examined using Student Newman-Keuls tests.

RESULTS

Neuron classification

We recorded extracellular activity from 50 gustatory neurons in the geniculate ganglion. Hierarchical cluster analysis grouped neurons based on the similarity of responses to the four taste stimuli at the standard 25°C temperature as shown in the dendrogram in Fig. 2. Groups of neurons with a high degree of similarity were closest to 0 on the scale. Based on the cluster analysis, six groups of neurons were identified: two clusters of specialist neurons that responded primarily to their best stimulus, and four clusters of generalist neurons that responded

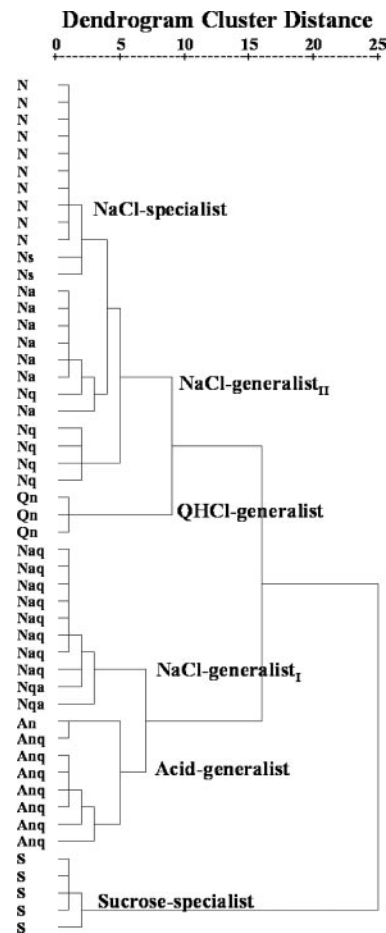


FIG. 2. Dendrogram showing the results of the hierarchical cluster analysis. Next to each neuron is the symbol of the taste stimulus (S, 0.05 M sucrose; N, 0.1 NaCl; A, 0.01 M citric acid, and Q, 0.02 M QHCl) that evoked the best response, followed by the symbol(s) of other taste stimuli that evoked a response $\geq 30\%$ of the best response.

robustly to multiple taste stimuli. The response profiles of all 50 neurons, grouped on the basis of the cluster analysis and arranged within each group by the taste stimulus that evoked the greatest response at the standard temperature, are shown in Fig. 3. Although there was considerable variability in firing rates, response patterns were highly consistent within each neuron group.

Specifically, *Sucrose-specialist neurons* ($n = 5$) responded robustly to 0.5 M sucrose and were weakly activated, if at all, by other taste stimuli.

NaCl-specialist neurons ($n = 12$) responded robustly to 0.1 M NaCl and little, if at all, to any other taste. Based on the Poisson distribution, two neurons responded weakly to sucrose, but were included in the cluster, as they were more like NaCl specialists than any other group.

NaCl-generalist neurons responded best to NaCl and also responded to at least one other taste. As shown in the dendrogram, two distinct types of NaCl-generalist neurons were identified: NaCl-generalists_I and NaCl-generalists_{II}. NaCl-generalists_I neurons ($n = 10$) responded best to NaCl and also responded well to citric acid and QHCl. NaCl-generalists_{II} neurons ($n = 12$) responded best to NaCl and also responded to one other taste. Of these 12 neurons, 8 responded second best to citric acid, whereas 4 responded second best to QHCl.

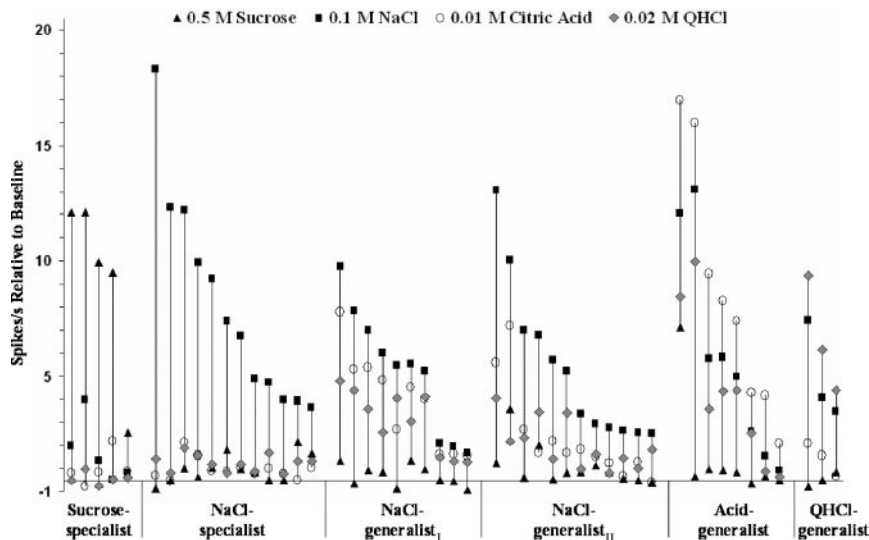


FIG. 3. Responses of each neuron to 0.05 M sucrose, 0.1 NaCl, 0.01 M citric acid, 0.02 M QHCl at the standard 25°C temperature. Within each group, neurons were arranged by the response (spikes/s relative to baseline) to the best stimulus in descending order from left to right.

NaCl-generalist_{II} neurons were closely related to NaCl-specialist neurons; however, they differed from specialists in that the response evoked by either citric acid or QHCl was $\geq 30\%$ of the response evoked by NaCl.

Acid-generalist neurons ($n = 8$) responded best to citric acid. Similar to NaCl-generalist_I, Acid-generalists also responded well to NaCl and QHCl and little to sucrose.

QHCl-generalist neurons ($n = 3$) responded best to QHCl. These neurons also responded well to NaCl, but little, if at all to citric acid or sucrose. Although we recorded from only a small number of QHCl-generalist neurons, it is clear from the cluster analysis that they were more closely related to the NaCl-generalist_{II} than to any other group.

Breadth of tuning

Mean H values for each neuron group at 10, 25, and 40°C are shown in Table 1. At all temperatures, Sucrose- and NaCl-specialist neurons were the most narrowly tuned groups. NaCl-generalist_{II} and QHCl-generalist neurons were moderately tuned, responding well to two of the four taste stimuli. NaCl-generalist_I and Acid-generalist neurons were broadly tuned, responding well to three of the four taste stimuli. One-way RM ANOVAs indicated that breadth of tuning was not affected by temperature in Sucrose-specialist, NaCl-specialist, NaCl-generalist_I, NaCl-generalist_{II}, or QHCl-generalist neurons. In contrast, there was a significant main effect of temperature on the breadth of tuning for Acid-generalist neurons [Table 1; $F(4,28) = 3.78$, $P < 0.05$] and post hoc analyses

showed that these neurons were less broadly tuned at 10 than at 25 or 40°C (P values < 0.05).

Thermal sensitivity and spontaneous neural activity

The numbers of neurons that responded to gradual cooling or warming are shown in Table 2. Approximately 30–35% of the 50 neurons responded to cooling, and only ~15–25% responded to warming. Interestingly, however, examination of the neuron groups revealed that the majority of neurons that responded to gradual temperature changes were from two specific groups (NaCl-specialist and Acid-generalist neurons) and that the thermal sensitivity of these two groups was within specific temperature ranges (10–25°C for NaCl-specialists) or depended on the adapted temperature (40°C for Acid-generalists). Figure 4 shows raw electrophysiological traces from a representative NaCl-specialist (A and B) and an Acid-generalist (E) that responded to gradual temperature changes as well as from representative NaCl-generalist_I (C) NaCl-generalist_{II} (D), and Sucrose-specialist (F) neurons that were unresponsive to thermal stimuli. For the sucrose specialist, warming from 25 to 40°C is shown for comparison with work by Ogawa et al. (1968) examining responses to warming from 20 to 40°C.

SUCROSE-SPECIALIST NEURONS. Sucrose-specialists were unresponsive to gradual cooling or warming of the tongue (see Fig. 4F) as indicated by two-way RM ANOVA, and their baseline firing rate was similar after 4-min adaptation to 10, 25, or 40°C (Table 3) as revealed by one-way RM ANOVA.

TABLE 1. Breadth of tuning, indicated by H-values, for each neuron group at 10°C, 25°C, or 40°C

Neuron Type	10°C	25°C			40°C
		1	2	3	
Sucrose specialist	0.21 ± 0.13	0.24 ± 0.06	0.20 ± 0.08	0.20 ± 0.10	0.23 ± 0.12
NaCl specialist	0.28 ± 0.06	0.23 ± 0.04	0.24 ± 0.07	0.21 ± 0.06	0.16 ± 0.04
NaCl generalist _I	0.69 ± 0.05	0.73 ± 0.04	0.72 ± 0.05	0.73 ± 0.03	0.71 ± 0.05
NaCl generalist _{II}	0.56 ± 0.07	0.54 ± 0.07	0.59 ± 0.04	0.53 ± 0.05	0.60 ± 0.05
Acid generalist	0.59 ± 0.05*	0.71 ± 0.06	0.69 ± 0.04	0.72 ± 0.03	0.73 ± 0.04
QHCl generalist	0.40 ± 0.08	0.62 ± 0.05	0.56 ± 0.05	0.61 ± 0.09	0.40 ± 0.19

Values are means ± SE. 1, 2, and 3 are beginning, middle, and end respectively. *, significantly different from H-values at all other temperatures.

TABLE 2. Number of neurons responding to cooling and warming from adapted temperatures as indicated by the Poisson distribution

	Cooling		Warming	
	40°C–25°C	25°C–10°C	10°C–25°C	25°C–40°C
Total No. of Responsive Neurons	17/50	15/50	11/50	6/50
Neuron Type				
Sucrose-specialist	0/5	0/5	0/5	0/5
NaCl-specialist	2/12	9/12	9/12	3/12
NaCl-generalist _I	2/10	2/10	1/10	2/10
NaCl-generalist _{II}	3/12	2/12	0/12	0/12
Acid-generalist	8/8	1/8	1/8	1/8
QHCl-generalist	2/3	1/3	0/3	0/3

NaCl-SPECIALIST NEURONS. Fig. 5 shows the mean responses (relative to baseline) of NaCl-specialist neurons to gradual cooling and warming of the tongue after adaptation to 10, 25, and 40°C. The *top panel* shows responses to cooling and warming between 10 and 25°C, and the *bottom panel* shows responses to cooling and warming between 25 and 40°C. Two-way RM ANOVA revealed a significant main effect of temperature on firing rate [$F(3,33) = 5.61, P < 0.01$] and a significant interaction between temperature and time [$F(9,99) = 3.67, P < 0.001$]. Post hoc analyses revealed that cooling from 25 to 10°C evoked a significant increase in firing during the last 5 s of stimulation ($P < 0.01$; see also Fig. 4A), whereas warming from 10 to 25°C significantly decreased firing during the last 5 s ($P < 0.05$; see also Fig. 4B). In both conditions, firing rate changed from baseline only after the temperature had increased or decreased by $\sim 10^\circ\text{C}$. However, the thermal sensitivity of NaCl-specialist neurons was restricted to the 10 to 25°C temperature range, as NaCl-specialists were unresponsive to cooling or warming in the 25 to 40°C range.

One-way RM ANOVA also revealed a significant main effect of temperature on baseline firing rate of NaCl-specialist

neurons after 4-min adaptation to 10, 25, or 40°C [$F(4,44) = 3.07, P < 0.05$] and post hoc analyses showed that spontaneous activity was greater at 10 than at 40°C ($P < 0.01$).

NaCl-GENERALIST_I NEURONS. Two-way RM ANOVA revealed a significant main effect of temperature on firing rate [$F(3,27) = 6.56, P < 0.01$] and a significant interaction between temperature and time [$F(9,81) = 2.29, P < 0.05$]. However, post hoc analyses showed no effect of cooling or warming from any adapted temperature at any time point (see Fig. 4C). In fact, the effect of thermal stimulation likely was attributable to the effect of adapted temperature on baseline firing rate [Table 3; $F(4,36) = 2.83, P < 0.05$]. Post hoc analyses showed that baseline activity was less at 10°C than at either 25 or 40°C (all P values < 0.05). Thus although baseline firing activity was decreased after 4-min adaptation to 10°C, NaCl-generalist_I were unresponsive to gradual cooling or warming of the tongue from the three adapted temperatures.

NaCl-GENERALIST_{II} NEURONS. NaCl-generalist_{II} also were unresponsive to gradual cooling or warming of the tongue (see Fig. 4D), as revealed by two-way RM ANOVA. However, unlike NaCl-generalist_I, the baseline firing rate of NaCl-

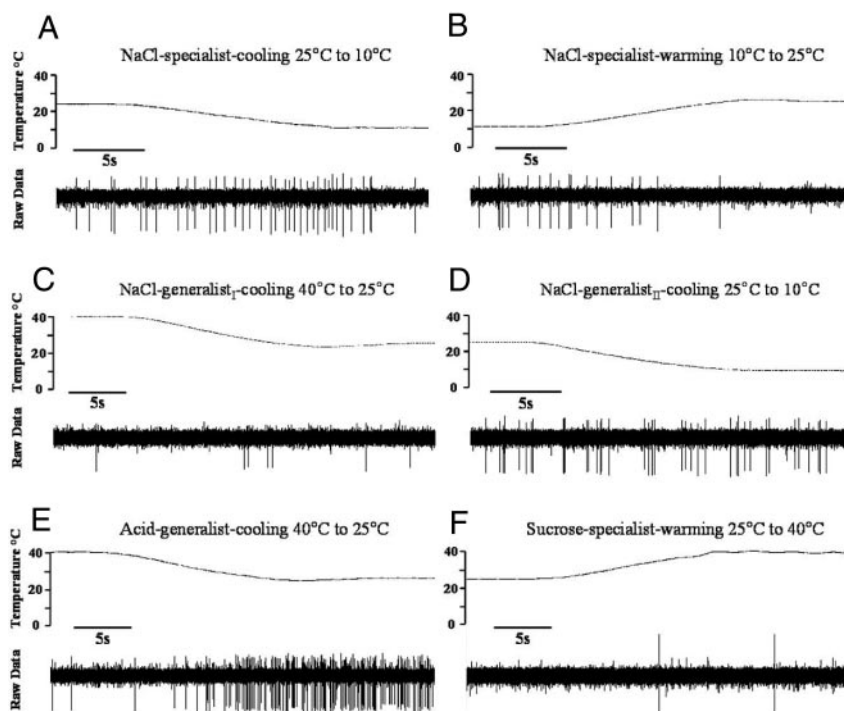


FIG. 4. Raw electrophysiological traces from individual gustatory neurons in response to gradual cooling and warming from adapted temperatures. The same NaCl-specialist neuron responding to cooling from 25 to 10°C (A) and warming from 10 to 25°C (B); C: NaCl-generalist_I neuron unresponsive to cooling from 40 to 25°C; D: NaCl-generalist_{II} neuron unresponsive to cooling from 25 to 10°C; E: acid-generalist neuron responding to cooling from 40 to 25°C; and F: Sucrose-specialist neuron unresponsive to warming from 25 to 40°C.

TABLE 3. Baseline spontaneous firing rates (spikes/s) of each neuron group after 4-min adaptation at 10°C, 25°C, or 40°C

Neuron Type	10°C	25°C			40°C
		1	2	3	
Sucrose-specialist	0.12 ± 0.05	0.23 ± 0.09	0.19 ± 0.05	0.17 ± 0.04	0.24 ± 0.09
NaCl-specialist	1.30 ± 0.38	1.00 ± 0.33	1.00 ± 0.34	0.97 ± 0.42	0.62 ± 0.25
NaCl-generalist _I	0.33 ± 0.10	0.70 ± 0.20	0.74 ± 0.24	0.85 ± 0.21	0.83 ± 0.32
NaCl-generalist _{II}	1.17 ± 0.60	1.21 ± 0.54	1.32 ± 0.53	1.48 ± 0.55	1.22 ± 0.61
Acid-generalist	0.14 ± 0.04	0.60 ± 0.26	0.66 ± 0.26	0.82 ± 0.35	0.63 ± 0.31
QHCl-generalist	0.28 ± 0.17	0.52 ± 0.45	0.47 ± 0.21	0.58 ± 0.47	0.16 ± 0.14

Values are means ± SE. 1, 2, and 3 are beginning, middle, and end respectively.

generalist_{II} after 4-min adaptation was not significantly different at 10, 25, or 40°C as revealed by one-way RM ANOVA (Table 3).

ACID-GENERALIST NEURONS. Figure 6 shows the mean responses of Acid-generalists during gradual cooling and warming of the tongue after adaptation to 10, 25, and 40°C. The *top panel* shows responses to cooling and warming from 10 to 25°C, and the *bottom panel* shows responses to cooling and warming from 25 to 40°C. Two-way RM ANOVA revealed a significant main effect of temperature [$F(3,21) = 6.55, P < 0.01$], a significant main effect of time [$F(3,21) = 5.05, P < 0.01$], and a significant interaction between temperature and time [$F(9,63) = 7.33, P < 0.001$]. Post hoc analyses showed that cooling from 40 to 25°C (but not from 25 to 10°C) evoked

a significant increase in firing rate over the last 10 s of stimulation (P values < 0.05 ; see also Fig. 4E). Thus Acid-generalist neurons were responsive only to cooling and only after adaptation at 40°C.

A one-way RM ANOVA revealed that adapted temperature influenced baseline firing rate of Acid-generalist neurons [Table 3; $F(4,28) = 3.35, P < 0.05$] and post hoc analyses showed that baseline activity was less after at 10°C than at either 25 or 40°C (all P values < 0.05).

QHCl-GENERALIST NEURON. QHCl-generalist neurons were unresponsive to cooling or warming of the tongue, but this was based on a small number of neurons. Two-way RM ANOVA revealed no differences in firing rate during temperature changes. Moreover, one-way RM ANOVA revealed no differ-

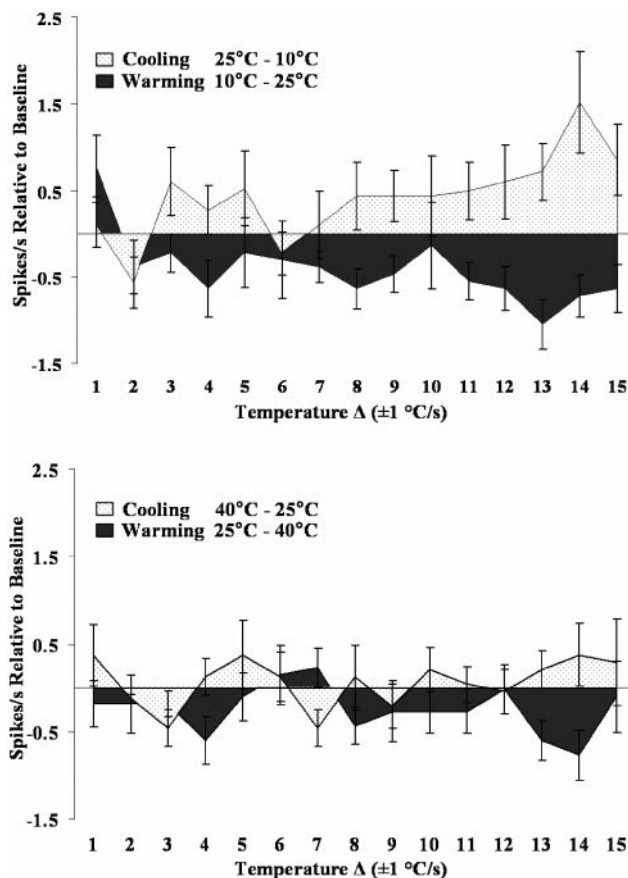


FIG. 5. Mean responses (spikes/s relative to baseline) of NaCl-specialist neurons to gradual warming and cooling at 1°C/s for 15 s from 10 to 25°C (*top*) and 25 to 40°C (*bottom*).

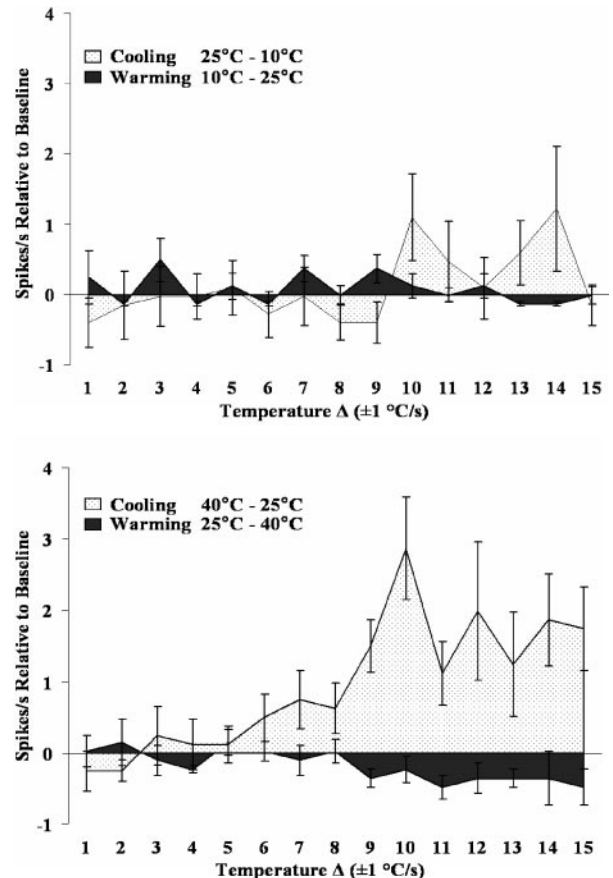


FIG. 6. Mean responses (spikes/s relative to baseline) of Acid-generalist neurons to gradual warming and cooling at 1°C/s for 15 s from 10 to 25°C (*top*) and 25 to 40°C (*bottom*).

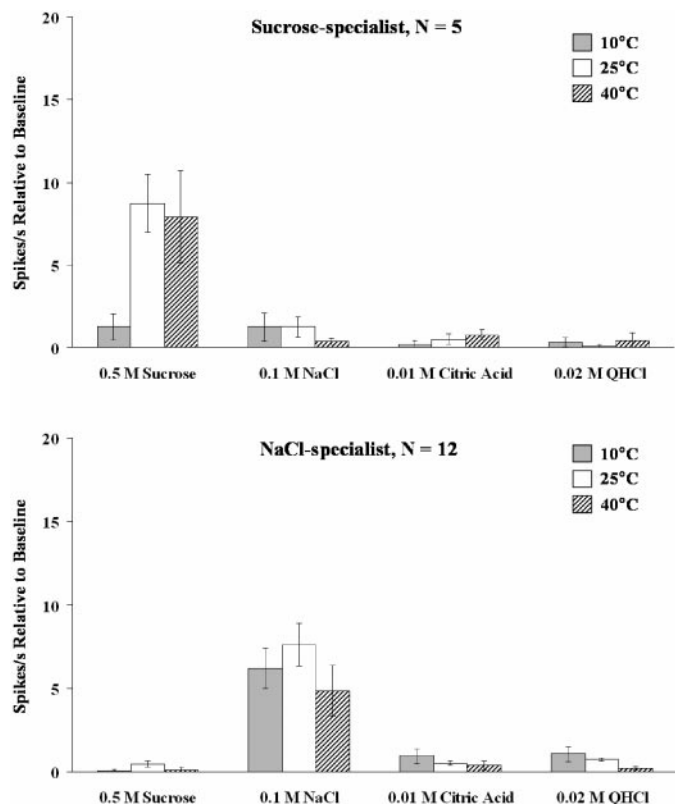


FIG. 7. Mean responses (spikes/s relative to baseline) to the four basic taste stimuli at 10°C (■), 25°C (□), or 40°C (▨) by Sucrose-specialist neurons (*top*) and NaCl-specialist neurons (*bottom*).

ences in firing rate after 4-min adaptation at 10, 25, or 40°C (Table 3).

Temperature-taste interaction

Figures 7–9 show the mean responses by each of the six neuron groups to the four basic taste stimuli after 4-min adaptation to 10, 25, and 40°C.

SUCROSE-SPECIALIST NEURONS. Two-way RM ANOVA revealed significant main effects of taste [$F(3,12) = 11.54, P < 0.001$] and temperature [$F(2,8) = 7.82, P < 0.05$] and a significant interaction between temperature and taste [$F(6,24) = 7.03, P < 0.001$] on firing rates to taste stimuli by Sucrose-specialists (Fig. 7, *top*). Post hoc analyses showed that at both 25 and 40°C, the sucrose response was significantly greater than responses to all other taste stimuli (all P values < 0.001). However, at 10°C, sucrose specialists responded similarly to all four stimuli. Furthermore, the Sucrose-response at 10°C was significantly less than those at 25 or 40°C (all P values < 0.001), which were not different from each other.

NaCl-SPECIALIST NEURONS. Two-way RM ANOVA revealed a significant main effect of taste [$F(3,33) = 26.53, P < 0.001$] on firing rates to taste stimuli by NaCl-specialists (Fig. 7, *bottom*) with the NaCl response being significantly greater than responses to all other taste stimuli (all P values < 0.001). There was no effect of temperature and no interaction between temperature and taste; thus NaCl-specialist neurons retained their specialist characteristics at all temperatures.

NaCl-GENERALIST_I NEURONS. Two-way RM ANOVA revealed significant main effects of taste [$F(3,27) = 32.92, P < 0.001$] and temperature [$F(2,18) = 5.996, P < 0.01$] and a significant interaction between taste and temperature [$F(6,54) = 4.44, P < 0.01$] on firing rates to taste stimuli by NaCl-generalists_I (Fig. 8, *top*). Post hoc analyses showed that, at 25°C, the NaCl response was significantly greater than responses to all other tastants (all P values < 0.05). At 10°C, the NaCl response was significantly greater than the sucrose response ($P < 0.01$) but was not different from either citric acid or QHCl responses. At 40°C, the NaCl response was significantly greater than QHCl and sucrose responses (all P values < 0.001) but was not different from the citric acid response ($P = 0.061$). The response evoked by NaCl at 10°C was significantly less than those at 25 or 40°C (all P values < 0.001), which were not different from each other ($P = 0.051$); thus adapted temperature greatly influenced the overall response pattern of NaCl-generalists_I.

In regard to other taste stimuli, the citric acid response at 10°C was significantly greater than responses to sucrose and QHCl (all P values < 0.05) but was not different from the response to NaCl. At 25°C, citric acid and QHCl responses were not different from each other, but both were significantly greater than the sucrose response ($P < 0.001$). At 40°C, the citric acid response was significantly greater than QHCl and sucrose responses (all P values < 0.05), and the QHCl response was significantly greater than the sucrose response ($P < 0.001$). The citric acid response was significantly less at 10 than at 40°C ($P < 0.001$), and the QHCl response was significantly less at 10 than at either 25 or 40°C (P values

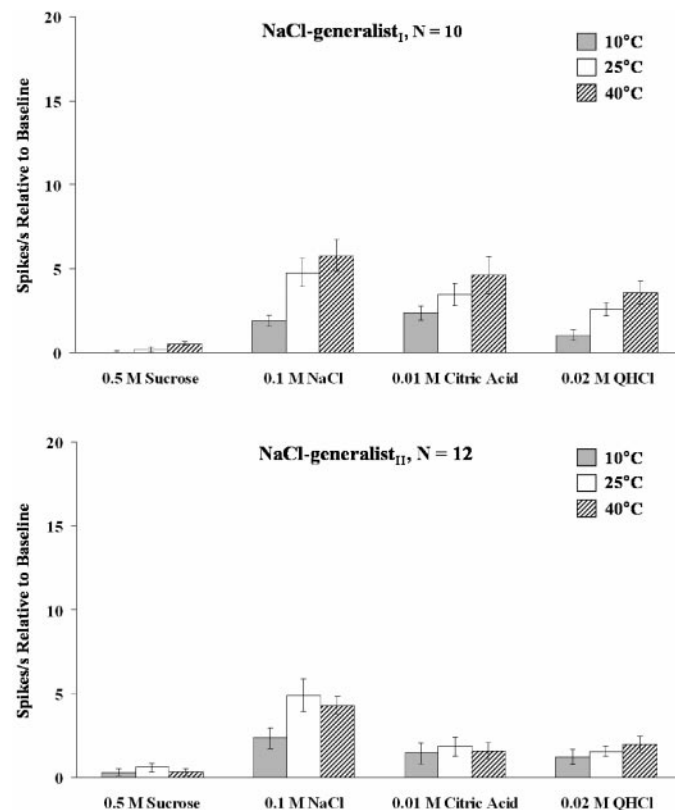


FIG. 8. Mean responses (spikes/s relative to baseline) to the four basic taste stimuli at 10°C (■), 25°C (□), or 40°C (▨) by NaCl-generalist_I neurons (*top*) and NaCl-generalist_{II} neurons (*bottom*).

<0.05). There were no differences in responses evoked by sucrose at any temperature.

NaCl-GENERALIST_{II} NEURONS. Two-way RM ANOVA revealed a significant main effect of taste [$F(3,33) = 19.64, P < 0.001$] and a significant interaction between temperature and taste [$F(6,66) = 4.87, P < 0.001$] on firing rates to taste stimuli by NaCl-generalists_{II} (Fig. 8, *bottom*). Post hoc analyses showed that at 25 and 40°C, the NaCl response was significantly greater than responses to all other taste stimuli (P values <0.001). At 10°C, the NaCl response was significantly greater than the sucrose response ($P < 0.001$) but was not different from responses to citric acid or QHCl. The response evoked by NaCl at 10°C was significantly less than those at 25 or 40°C (P values <0.001), which were not different from each other.

In regard to other taste stimuli, NaCl-generalist_{II} neurons responded similarly to citric acid and QHCl at 10, 25, and 40°C. However, the response to citric acid was greater than that to sucrose at 10, 25, and 40°C (all P values < 0.05), whereas the response to QHCl was greater than that to sucrose only at 40°C ($P < 0.01$). Sucrose was an ineffective stimulus for NaCl-generalist_{II} neurons at all temperatures.

ACID-GENERALIST NEURONS. Two-way RM ANOVA revealed significant main effects of taste [$F(3,21) = 14.197, P < 0.001$] and temperature [$F(2,14) = 10.43, P < 0.01$] and a significant interaction between temperature and taste [$F(6,42) = 7.39, P < 0.001$] on firing rates to taste stimuli by Acid-generalists (Fig. 9A). Post hoc analyses showed that at 25 and 40°C, the citric acid responses were significantly greater than responses to all other stimuli (all P values < 0.05). At 10°C, the citric

acid response was significantly greater than the sucrose response ($P < 0.05$) but was not different from responses to NaCl or QHCl. Unlike other neuron types, Acid-generalists responded linearly to their best stimulus, citric acid, with increasing temperature. The citric acid response at 10°C was significantly less than that at 25°C ($P < 0.01$), and the response at 25°C was significantly less than that at 40°C ($P < 0.001$).

In regard to other taste stimuli, the NaCl response at 25°C was significantly greater than the sucrose response ($P < 0.01$), whereas the NaCl response at 40°C was significantly greater than QHCl and sucrose responses (all P values <0.01). Moreover, the QHCl response at 40°C was significantly greater than the sucrose response ($P < 0.001$). NaCl responses at 10 and 25°C were similar ($P = 0.058$) but were both significantly less than the NaCl response at 40°C (all P values <0.001). The QHCl response at 10°C was significantly less than that at 40°C ($P < 0.01$). There were no differences in responses evoked by sucrose at any temperature.

QHCl-GENERALIST NEURONS. Two-way RM ANOVA revealed a significant main effect for taste [$F(3,6) = 17.193, P < 0.01$] but no effect of temperature and no interaction between temperature and taste on firing rates to taste stimuli by QHCl-generalists (Fig. 9, *bottom*). Post hoc analysis showed that responses to QHCl and NaCl were comparable and responses to both were significantly greater than responses to citric acid and sucrose (all P values < 0.01).

Recording stability

Responses to each of the four taste stimuli were evaluated following adaptation to the standard 25°C temperature at the beginning, middle, and end of the protocols (see Fig. 1) for each neuron group to assess the stability of the recording. Two-way RM ANOVA revealed no change in the response profiles (see Table 4) or in the response magnitude to any taste stimuli for any neuron group (Table 4). Additionally, after 4-min adaptation to 25°C at the beginning, middle, and end of the protocols, there were no differences in baseline firing rates (Table 3) or in breadth of tuning (Table 1).

DISCUSSION

We used extracellular single-cell recording procedures to characterize the responses of 50 neurons from the rat geniculate ganglion to lingual taste and thermal stimulation at three adapted tongue temperatures. Based on their response profiles to the four basic taste stimuli at the standard 25°C temperature, the neurons separated into two narrowly tuned and four broadly tuned neuron groups, designated specialists and generalists, and further categorized by the “best” stimulus eliciting the largest frequency response according to the nomenclature used by Lundy and Contreras (1999). These six neuron groups were similar in kind and relative frequency to those identified in prior investigations (Boudreau et al. 1983; Lundy and Contreras 1999; Sollars and Hill 2005). The only difference between studies was that we found three groups of “NaCl best” neurons that responded robustly to the NaCl stimulus instead of two. This difference seems to rest with the sour stimulus as HCl was used in our previous studies (Frank et al. 1983; Lundy and Contreras 1999) and citric acid was used in the present study.

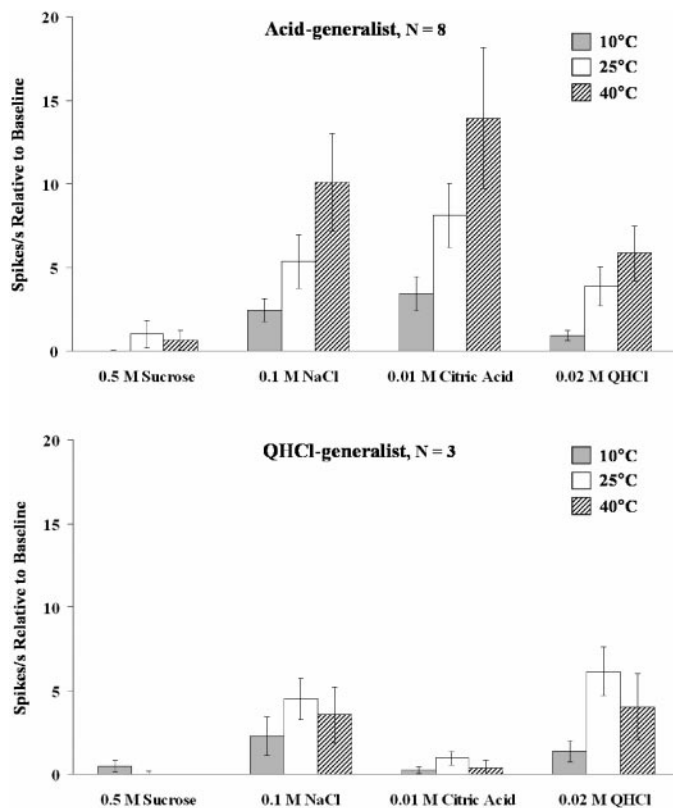


FIG. 9. Mean responses (spikes/s relative to baseline) to the four basic taste stimuli at 10°C (■), 25°C (□), or 40°C (▨) by Acid-generalist neurons (*top*) and QHCl-generalist neurons (*bottom*).

TABLE 4. Responses by each neuron group to each of the four tastants at the standard 25°C temperature at the beginning (1), middle (2), and end (3) of the protocol

	Sucrose-Specialist	NaCl-Specialist	NaCl-Generalist _I	NaCl-Generalist _{II}	Acid-Generalist	QHCl-Generalist
Sucrose-1	8.7 ± 1.8	0.5 ± 0.2	0.2 ± 0.1	0.6 ± 0.3	1.0 ± 0.8	0.0 ± 0.2
Sucrose-2	8.8 ± 2.1	0.3 ± 0.2	0.2 ± 0.1	0.3 ± 0.2	0.3 ± 0.3	0.0 ± 0.2
Sucrose-3	7.2 ± 1.0	-0.1 ± 0.1	0.2 ± 0.2	0.4 ± 0.2	0.0 ± 0.1	-0.2 ± 0.1
NaCl-1	1.2 ± 0.6	7.6 ± 1.3	4.8 ± 0.8	4.9 ± 1.0	5.4 ± 1.6	4.5 ± 1.2
NaCl-2	0.7 ± 0.4	7.4 ± 1.5	4.3 ± 0.8	4.2 ± 0.8	5.5 ± 1.6	4.9 ± 0.8
NaCl-3	0.9 ± 0.7	7.2 ± 1.3	4.3 ± 0.8	4.5 ± 0.9	5.6 ± 1.7	4.1 ± 1.6
Citric acid-1	0.5 ± 0.3	0.5 ± 0.1	3.4 ± 0.6	1.8 ± 0.6	8.1 ± 1.9	0.9 ± 0.4
Citric acid-2	0.7 ± 0.2	0.5 ± 0.3	3.3 ± 0.7	1.7 ± 0.5	7.8 ± 1.7	0.9 ± 0.5
Citric acid-3	0.4 ± 0.2	0.1 ± 0.2	3.5 ± 0.7	1.2 ± 0.3	8.2 ± 2.1	1.0 ± 0.6
QHCl-1	0.1 ± 0.1	0.7 ± 0.1	2.6 ± 0.4	1.5 ± 0.3	3.8 ± 1.2	6.1 ± 1.5
QHCl-2	0.1 ± 0.1	1.2 ± 0.5	2.4 ± 0.5	2.3 ± 0.6	2.3 ± 0.6	5.7 ± 2.0
QHCl-3	0.6 ± 0.7	0.6 ± 0.2	2.4 ± 0.5	1.6 ± 0.4	3.1 ± 1.1	5.1 ± 1.9

Values are means ± SE.

HCl is a strong mineral acid, whereas citric acid is a naturally occurring weak acid. Both types of acids initiate sour taste transduction by increasing intracellular pH; however, mineral acids like HCl activate taste receptor cells by direct entry of H⁺ ions through proton channels in the plasma membrane, whereas weak acids cross the plasma membrane as neutral molecules and then dissociate to lower intracellular pH (Desimone 2001; Lyall et al. 2001, 2002a,b, 2004; Vinnikova et al. 2004). Consequently, HCl should have a greater influence on the distribution of neuron groups than citric acid. Indeed, this was the case as Lundy and Contreras (1999) reported that with HCl, 35–50% of the total sample consisted of acid responsive neurons (Lundy and Contreras 1999), whereas only 16% of the neurons in present study consisted of Acid-generalist neurons with citric acid as the sour stimulus. Some of the neurons classified as NaCl-generalist_I in the present study might have been classified as acid generalists if HCl was the sour taste stimulus as in our previous study. Nevertheless, striking differences emerged between NaCl-generalist_I and Acid-generalist neurons in breadth of tuning and in responses to gradual cooling (see following text) that justifies classifying Acid-generalist, NaCl-generalist_I, and NaCl-generalist_{II} neurons as distinct groups. In short, temperature sensitivity of the

neuron groups provides another dimension by which to assess the cluster groupings.

To our knowledge, the present work is the first to demonstrate that the thermal sensitivity of geniculate ganglion neurons was limited to two specific neuron types and depended on adapted temperature. NaCl-specialists responded to both cooling and warming but only when the tongue was adapted to temperatures within a range of 10–25°C. In contrast, acid generalists responded only to cooling and only when the tongue was adapted to a warmer temperature of 40°C (see also Lundy and Contreras 1999; Ogawa et al. 1968). Moreover, when the tongue was cooled from 40 to 10°C at 1°C/s for three Acid-generalists, these neurons responded as expected, with progressively increasing firing rate that reached a peak of ~6 spikes/s above baseline at the lowest temperature (Fig. 10) (see also Lundy and Contreras 1999). Furthermore, adapted temperature influenced spontaneous firing rate in the opposite direction, as the spontaneous activity of NaCl-specialists was at its peak at 10°C, whereas that of acid generalists was at its nadir. Finally, adapted tongue temperature also influenced taste sensitivity and this effect also depended on neuron type. In sum, the present findings show that temperature modulates taste responsiveness and is itself a stimulus in specific types of peripheral gustatory neurons.

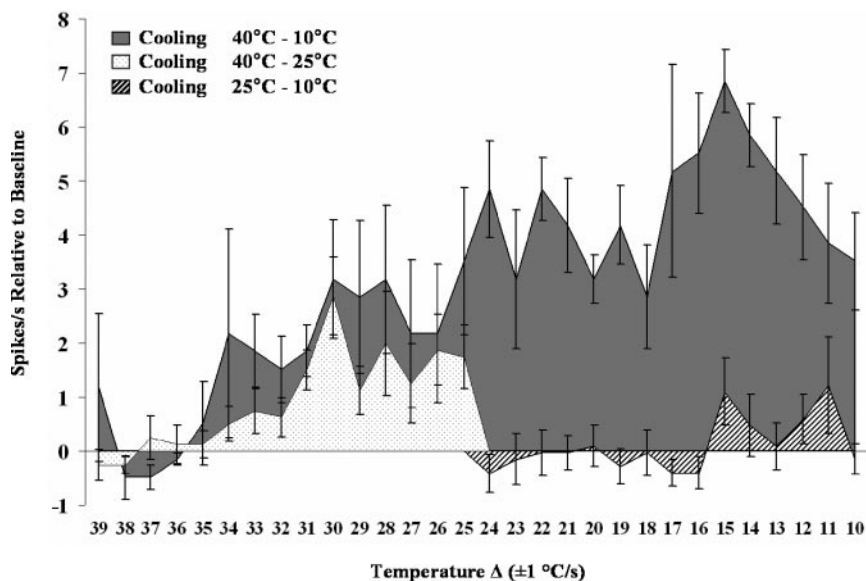


FIG. 10. Mean responses (spikes/s relative to baseline) by Acid-generalist neurons to gradual cooling at 1°C/s from 40 to 10, 40 to 25, and 25 to 10°C.

Thermal sensitivity

Two previous studies (Lundy and Contreras 1999; Ogawa et al. 1968) identified the thermal sensitivity of acid generalists; however, this is first study to show thermal sensitivity of NaCl-specialist neurons. NaCl-specialist neurons responded to cooling and warming over a relatively narrow temperature range, corresponding to room temperature and below; NaCl-specialists were unresponsive to gradual cooling from 40 to 25°C, which is consistent with our previous work (Lundy and Contreras 1999) demonstrating that NaCl-specialist neurons were unresponsive to cooling from an adapted temperature of 35°C.

Previous studies showed that single-fiber responses of the rat chorda tympani nerve to HCl and QHCl were positively correlated with their responses to sudden cooling from 40 to 20°C (Ogawa et al. 1968). Additionally, Acid-generalist neurons from the geniculate ganglion increased firing when cooled from 35 to 10°C by 1°C/s (Lundy and Contreras 1999), beginning to respond ~6 s after the onset of cooling when the temperature reached 29°C and continuing to fire above the baseline rate for the duration of cooling. However, these studies examined thermal sensitivity from only one adapted temperature. Thus in conjunction with previous studies (Lundy and Contreras 1999; Ogawa et al. 1968), our findings suggest that the robust response to cooling by acid-responsive neurons may require adaptation to temperatures $\geq 35^\circ\text{C}$ and be specific to cooling as neither cooling from 25 to 10°C nor warming from 25 to 40°C affected the firing rate of Acid-generalist neurons (Fig. 6). Our protocols do not allow us to rule out the possibility that, like NaCl specialists, Acid-generalist neurons are responsive to both cooling and warming but only from an adapted temperature of 40°C; however, we chose 40°C as the upper limit to avoid temperatures that could indirectly influence gustatory processing by stimulating heat-sensitive nociceptors (for review, see Green 2004).

In the present study, only NaCl-specialist and Acid-generalist neurons responded to thermal stimuli. Gradual warming of the tongue did not affect sucrose-specialist neurons and, consistent with our prior study (Lundy and Contreras 1999), NaCl-generalist neurons were unresponsive to thermal stimulation. Although Ogawa et al. (1968) reported that sudden warming from 20 to 40°C evoked robust increases in activity from sucrose-sensitive fibers in the chorda tympani nerve, this finding was specific to the hamster, as sucrose-sensitive fibers from the rat chorda tympani nerve were unresponsive to warming from 20 to 40°C. Our observations, therefore also are consistent with those of Ogawa et al. (1968) from rats.

There are some commonalities and differences between the responses of thermally sensitive neurons in the chorda tympani nerve that innervate fungiform taste buds and those in lingual trigeminal nerve the free nerve endings of which terminate within fungiform papillae surrounding taste buds (Whitehead et al. 1999). For both nerves, cooling of the tongue excited thermally sensitive fibers, whereas warming inhibited them (Lundy and Contreras 1994; Pittman and Contreras 1998). In previous studies (Lundy and Contreras 1995; Pittman and Contreras 1998), we found two groups of thermally sensitive lingual trigeminal fibers that responded to gradual cooling at a rate of 1°C/s from an adapted temperature of 35°C, similar to the conditions used in the present study. One group was more

responsive to gradual cooling at the upper end of the temperature range, and the other group was more responsive at the lower end (Lundy and Contreras 1995). These observations are somewhat akin to our results from acid generalists and NaCl specialists, respectively.

Despite the similarities, however, the lingual trigeminal nerve is more sensitive to thermal stimulation, as is apparent in the lower response threshold (3–4°C) and greater peak response magnitude (4–6 spikes/s) to gradual cooling in trigeminal fibers (Lundy and Contreras 1995), compared with the 6°C response threshold and one to two spikes/s peak response of NaCl-specialists and Acid-generalist neurons from the geniculate ganglion. Conversely, the chorda tympani nerve is more sensitive to taste stimuli, whereas lingual trigeminal fibers respond weakly, at best, to the basic taste stimuli (Pittman and Contreras 1998). Therefore there may be a reciprocal, and possibly interactive, relationship between gustatory and somatosensory afferent systems that innervate fungiform papillae. Thermal stimulation may influence mechanisms underlying taste intensity and quality coding, and taste stimulation may influence mechanisms underlying thermal sensation. The nature of this relationship is unclear, but transient receptor potential (TRP) channels (see Clapham 2002 2003 for review) expressed in taste receptor cells (Pérez et al. 2002) and lingual trigeminal nerve fibers innervating the fungiform papillae (Abe et al. 2005) may contribute to the initial response to taste and temperature stimulation.

Temperature–taste interaction

Few investigations have examined the effects of temperature on gustatory neuron responses to sapid stimuli with only one other study of geniculate ganglion neurons (Lundy and Contreras 1999). We found that geniculate ganglion neuron responses to basic taste stimuli differed as a function of adapted temperature. In general, narrowly tuned neuron groups retained the specificity of their responses. NaCl-specialists responded equally to 0.1 M NaCl at 10, 25, and 40°C (Fig. 7, *bottom*); this is consistent with our preliminary results (Lundy and Contreras 1999) showing NaCl-specialists to be equally responsive at 15, 25, and 35°C. Additionally, 5 of the 12 NaCl-specialists responded best to NaCl at 10°C, whereas 7 responded best at 25°C. These data are consistent with findings by Nakamura and Kurihara (1988) that there are two amiloride-sensitive fiber types in the chorda tympani nerve, one with a peak response to 0.1 M NaCl at 10°C and the other at 30°C. Sucrose-specialists responded equally to sucrose at both 25 and 40°C but were virtually unresponsive to sucrose at 10°C (Fig. 7, *top*). Thus this cool temperature may have temporarily suppressed the receptor/transduction mechanism for sweet taste, which recovered quickly when the temperature returned to 25°C. These results also suggest that low temperatures may compromise sweet taste perception (see also Green and Frankmann 1988).

Of the broadly tuned neuron groups, the pattern of responses was least affected by temperature in Acid-generalists. In fact, their acid > NaCl > QHCl profile was unaffected by adapted temperature (Fig. 9, *top*) despite the progressive increase in responses to all taste stimuli except sucrose with increasing temperature. This response pattern is similar to that reported in our earlier study for HCl-generalists adapted to a smaller range of temperatures (15–35°C) (Lundy and Contreras 1999). In

contrast, NaCl-generalist_{II} neurons responded best to NaCl at 25 and 40°C but equally to NaCl, citric acid, and QHCl at 10°C. Finally, NaCl-generalist_I neurons responded best to NaCl only at 25°C and equally to NaCl and citric acid at 10 and 40°C. However, the effect of temperature on acid and NaCl taste perception is more difficult to predict as, despite the temperature-dependent shift in response profiles in both groups of NaCl-generalist neurons, profiles remained stable in both NaCl-specialists and Acid-generalists.

Conclusion

Peripheral coding mechanisms of sweet, salt, sour, and bitter taste have generally been examined independently of the influence of temperature. In fact, most in vivo electrophysiological investigations of the peripheral taste system apply taste stimuli at room temperature. We identified six neuron groups in the rat geniculate ganglion, classified based on their responsiveness to the four basic tastes and separated into two major classes of narrowly tuned and broadly tuned neurons, and examined responses across a range of temperatures. These groups were distinguished, not only by their response profiles (Boudreau et al. 1983; Contreras and Lundy 2000; Lundy and Contreras 1999; Sollars and Hill 2005), but also by their responses to thermal stimuli and by the effect of adapted temperature on responses to the basic taste stimuli. Adapted temperature selectively influenced responses to the four basic taste stimuli in the two broadly tuned groups of NaCl-generalist neurons. In addition, Acid-generalists and NaCl-specialists were thermally sensitive, responding to slow cooling or to both cooling and warming, respectively. These responses depended on adapted temperature as Acid-generalists responded to cooling only when adapted to temperatures approximating body temperature, whereas NaCl-specialists responded to warming and cooling but only when adapted to temperatures at or below room temperature.

The fact that taste neurons respond to thermal stimuli raises questions about the nature of the sensory message being relayed to the brain. Temperature may merely modulate taste sensitivity, or the thermal stimulus may simulate taste (Cruz and Green 2000). We are aware of no studies that have addressed these issues in rats; however, in humans, temperature influences taste intensity and quality perception, and thermal stimulation itself appears to elicit taste sensations (Cruz and Green 2000). In the present study, cooling activated both Acid-generalists and NaCl-specialists. Although highly speculative, the activity elicited by cooling in Acid-generalists and NaCl-specialists may correspond with the thermal sour and salty tastes reported by humans. Similar to results from a previous study that examined chorda tympani fibers in rats (Ogawa et al. 1968), we found that warming was without effect on sucrose-specialist neurons. It is possible that warming may be more effective on the palate where sweet sensitivity is more prominent in rats (Nejad 1986), and ongoing studies are addressing this issue. Finally, the observation that response selectivity of NaCl-specialists for NaCl was uncompromised by adapted temperature suggests that the ability of NaCl-specialists to respond to NaCl across a wide range of temperatures may ensure the detection and ingestion of NaCl, which is essential for survival.

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