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Response Latency to Lingual Taste Stimulation Distinguishes Neuron Types Within the Geniculate Ganglion

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Breza JM, Nikonov AA, Contreras RJ. Response latency to lingual taste stimulation distinguishes neuron types within the geniculate ganglion. *J Neurophysiol* 103: 1771–1784, 2010. First published January 27, 2010; doi:10.1152/jn.00785.2009. The purpose of this study was to investigate the role of response latency in discrimination of chemical stimuli by geniculate ganglion neurons in the rat. Accordingly, we recorded single-cell 5-s responses from geniculate ganglion neurons ($n = 47$) simultaneously with stimulus-evoked summated potentials (electrogustogram; EGG) from the anterior tongue to signal when the stimulus contacted the lingual epithelium. Artificial saliva served as the rinse solution and solvent for all stimuli [(0.5 M sucrose, 0.03−0.5 M NaCl, 0.01 M citric acid, and 0.02 M quinine hydrochloride (QHCl)], 0.1 M KCl as well as for 0.1 M NaCl +1 μM benzamil. Cluster analysis separated neurons into four groups (sucrose specialists, NaCl specialists, NaCl/QHCl generalists and acid generalists). Artificial saliva elevated spontaneous firing rate and response frequency of all neurons. As a rule, geniculate ganglion neurons responded with the highest frequency and shortest latency to their best stimulus with acid generalist the only exception. For specialist neurons and NaCl/QHCl generalists, the average response latency to the best stimulus was two to four times shorter than the latency to secondary stimuli. For NaCl-specialist neurons, response frequency increased and response latency decreased systematically with increasing NaCl concentration; benzamil significantly decreased NaCl response frequency and increased response latency. Acid-generalist neurons had the highest spontaneous firing rate and were the only group that responded consistently to citric acid and KCl. For many acid generalists, a citric-acid-evoked inhibition preceded robust excitation. We conclude that response latency may be an informative coding signal for peripheral chemosensory neurons.

INTRODUCTION

Based on numerous electrophysiological single-cell recording studies, it is generally acknowledged that the mammalian peripheral gustatory system is divisible into narrowly and broadly tuned neuron groups (Boudreau 1983; Breza et al. 2006, 2007; Fishman 1957; Frank 1974; Frank et al. 1983; Lundy and Contreras 1999; Ninomiya and Funakoshi 1988; Ogawa et al. 1968; Pfaffmann 1955). Narrowly tuned neurons respond selectively to a single class of chemical stimuli. For example in the rat chorda tympani nerve, sucrose-specialist neurons respond robustly to sweet solutions and little if at all to the other basic taste stimuli. Likewise for NaCl-specialist neurons, which respond selectively to Na^+ solutions. In contrast, broadly tuned neurons respond to more than one class of chemical stimuli. Acid-generalist neurons from the rat chorda tympani nerve respond best to citric acid and also respond well to salt and bitter stimuli. In general, the breadth of tuning of individual chemosensory neurons has been determined by analysis of spike frequency responses to chemical stimulation over relatively long time intervals on the order of 5–20 s (Breza et al. 2006, 2007; Geran and Travers 2006; Lundy and Contreras 1999; Smith and Travers 1979; Sollars and Hill 2005). This greatly exceeds the minimum time it takes a rat to discriminate stimulus quality and intensity, which has been found to be within 600 ms after stimulus contact with the receptors (Halpern and Tapper 1971). Therefore there is a temporal disconnect between analysis of firing rate in peripheral chemosensory neurons and behavioral taste discrimination.

Thus a potential concern with prior electrophysiological investigations, ours included, is the absence of an accurate reference signal indicating the time when the stimulus contacts the receptive field. Without a reliable reference signal, investigators must estimate when the response begins by a change in firing rate and include an extended recording interval to insure sufficient impulse data for analysis. The traditionally used procedure has, on the one hand, been invaluable in providing insight into mammalian encoding of gustatory information. On the other hand, it has also been limiting to analysis of excitatory responses over time domains of dubious behavioral relevance, and possibly missing a more diverse range of responses (inhibition, OFF response, OFF-ON response, etc) seen in other sensory systems. The concern may be compounded by relying on stimulating parameters that do not take into account the physiological conditions naturally in play in terms of background temperature, rinse solution composition, and flow rate. Furthermore, considerable knowledge has recently been gained about taste-bud organization and function (Clapp et al. 2006; DeFazio et al. 2006; Finger et al. 2005; Herness and Chen 1997; Mueller et al. 2005; Tomchik et al. 2007; Vandenbeuch et al. 2008). Although the evidence is far from being clear, it appears that there may be direct (stimulus - receptor cell - afferent fiber) and converging (stimulus - receptor cell - presynaptic cell - afferent fiber) information processing pathways within the taste bud. Presumably, the former may be a slightly faster processing pathway than the latter. To tap more accurately into the information traffic occurring within the taste bud, there must be a reference point of stimulus contact for immediate analysis of spike response frequency and latency by narrowly and broadly tuned neurons.

Accordingly in the present investigation, we adopted a new measure of stimulus-evoked summated potential (electrogustogram, EGG) to determine the precise moment when the chemical stimulus contacts the lingual epithelium, while simultaneously recording single-cell responses from chemosensitive neurons in the rat geniculate ganglion. During recording, the

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tongue was adapted to 35°C artificial saliva (Hirata et al. 2005), instead of room temperature water, to protect lingual receptors and enhance spontaneous activity and neural responsiveness. Based on prior studies of taste-bud function, we stimulated the tongue with citric acid and KCl as a way to sort ganglion neurons into those that may receive input mostly from type II receptor cells from those that may receive input mostly from type III presynaptic cells (DeFazio et al. 2006; Huang et al. 2008; Tomchik et al. 2007). Finally, we tested a subset of the neurons with benzamil, a specific pharmacological blocker of epithelial sodium channels (ENaC), as a means to identify ganglion neurons that may receive input from type I receptor cells (Vandenbeuch et al. 2008). Our overall objective was to extend the functional characterization of rat geniculate ganglion neurons beyond that already obtained based solely on response frequency over an extended time period (Boudreau 1983; Breza et al. 2006, 2007; Lundy and Contreras 1999). Our analysis of breadth of tuning over short time intervals, determination of response latency to all stimuli, and characterization of neuron types with consideration to presumed input from unique cell types in the taste bud provide new insight into mammalian taste coding.

METHODS

Animals and surgery

Adult male Sprague-Dawley rats (Charles River Laboratories; $n = 19$) weighing 309–640 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_20). Rats were anesthetized with urethan (1.5 g/kg body wt) and, following a tracheostomy, 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 (Breza et al. 2006, 2007; 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.

Stimulus delivery and stimulation protocols

Solutions were presented to the anterior portion of the tongue using a custom-built computer-controlled fluid delivery system (R. Henderson, Florida State University) that 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 lick/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 allowed the temperature of the solutions to be held constant at 35 ± 0.3°C (mean ± SE) for the present investigation.

We tested each neuron's response to 5 s of stimulation with the basic taste stimuli—0.5 M sucrose, 0.1 M NaCl, 0.01 M citric acid, and 0.02 M quinine hydrochloride (QHCl)—and to 0.01 M KCl. Artificial saliva (Hirata et al. 2005) (0.015 M NaCl, 0.022 M KCl, 0.003 M $CaCl^2$, and 0.0006 $MgCl^2$; pH 5.8 ± 0.2) served as the rinse solution and solvent for the basic taste stimuli. All chemicals were reagent grade.

The tongue was adapted to 35°C artificial saliva, and responses to the basic taste stimuli and KCl were evaluated. Artificial saliva flowed continuously over the tongue for 90–120 s before and after the presentation of each taste stimulus. For a subset of neurons ($n = 28$), we assessed the effect of NaCl concentration (0.03, 0.1, 0.3, 0.5 M) on the response frequency and latency (Fig. 7). The response latency to 0.03 M NaCl was not included in analyses because not all neurons in each group fit the Poisson criteria for a significant response. Additionally, the effect of the epithelial sodium channel blocker, 1 μM benzamil HCl, on the response to 0.1 M NaCl, was assessed in the majority ($n = 25$) of this neural subset (Fig. 8). We used benzamil because it is a more potent (Treesukosol et al. 2007) and selective (Lundy and Contreras 1997) antagonist than amiloride. Responses (frequency and latency) to 0.1 M NaCl were measured before and after NaCl +1 μM benzamil HCl to evaluate the recording stability throughout the protocol (Fig. 8).

Recording techniques

EGG. The EGG was recorded in vivo with Ag/AgCl electrodes via saline-agar-filled capillary pipettes (Ø 100 μM, 0.15 M NaCl, 0.5% agar). We located the receptive field of the geniculate ganglion neuron by dotting a minute amount of chemical solution on the tongue from the bristle tips of a delicate artisan paintbrush. The EGG electrode and stimulus tube were placed near this region and remained unchanged until the stimulus protocol was completed. The signal was amplified (A-M System DC amplifier), digitized and stored on a PC. The signal served as a rapid response onset to the tested stimulus.

GENICULATE GANGLION UNIT RECORDING. Unit/few unit activity (generally 50–200 μV peak-to-peak amplitude) was recorded extracellularly. Low-impedance (1.0–1.5 MΩ) glass-insulated tungsten microelectrodes (tip: Ø 1 μM) were mounted on a stereotaxic micromanipulator (Siskiyou Design Instruments; Grants Pass, OR) and advanced downward from the dorsal surface of the ganglion. Neural activity was amplified (×10, 000; A-M Systems; Sequim, WA, bandpass 300–5,000 Hz), observed with an oscilloscope, optimized via a DAT recorder, digitized and stored on a PC.

Data analysis

Neuronal and EGG responses were monitored on-line and digitized using hardware and software (Spike 2; Cambridge Electronic Design, Cambridge, United Kingdom). Digitized responses were stored on computer for later analysis. Spike templates were formed on the basis of amplitude and waveform. The raw traces from four individual gustatory neurons types shown in Fig. 1 illustrate typical signal-to-noise ratios.

Spontaneous firing rate for each neuron was calculated as the average number of spike/second during the 5 s immediately prior to each stimulus. Response frequency was calculated as the difference between the spontaneous firing rate immediately prior to stimulation and the average number of spikes/s occurring during full 5-s period of chemical stimulation as well as for the first 2 and last 2 s of stimulation. Response latency was determined by the difference between the onset of the EGG waveform and the start of the neural response by means of vertical cursors in Spike 2. The Poisson distribution was used to detect significant changes ($P < 0.05$) from spontaneous firing rate during stimulation with each taste stimulus. In addition, the Poisson distribution was used to calculate the difference between the first second of 0.01 M citric acid presentation from baseline in acid-generalist neurons, to establish criteria for neurons that were inhibited or excited during this epoch.

Neurons were categorized based on their responses to the initial presentation of the four basic taste stimuli (0.5 M sucrose, 0.1 M NaCl, 0.01 M citric acid, and 0.02 M QHCl) by a hierarchal cluster analysis using Pearson's product-moment correlation coefficient (1 −

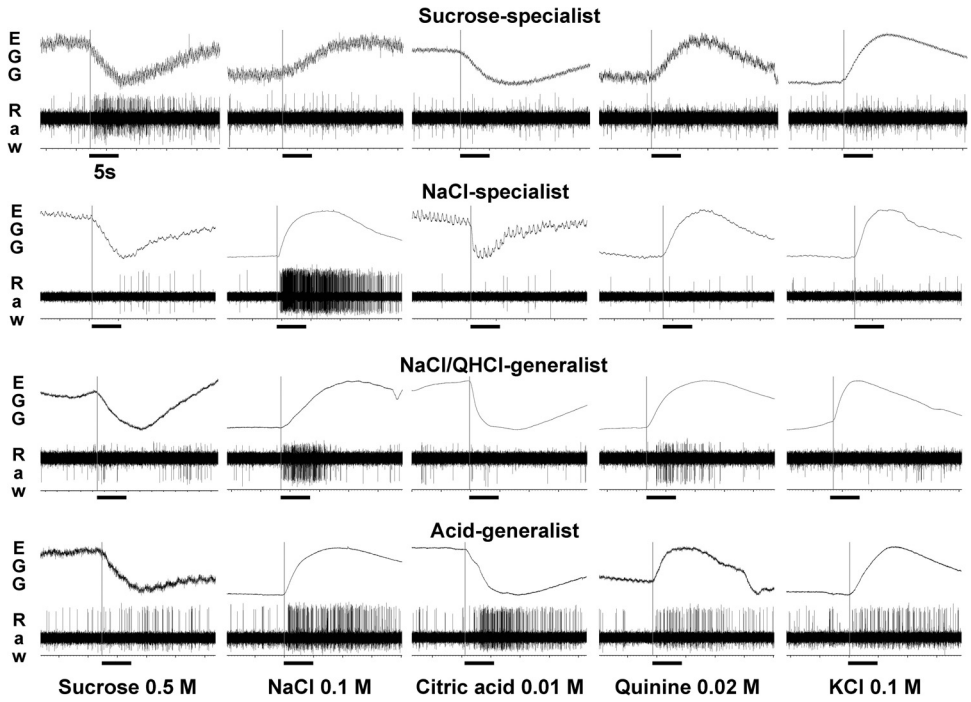

FIG. 1. Raw electrophysiological traces from individual gustatory neurons in response to 0.5 M sucrose, 0.1 M NaCl, 0.01 M citric acid, 0.02 M QHCl, and 0.1 M KCl.

r) and average-linking method between subjects (Statistica; StatSoft, Tulsa, OK).

Responses to the four 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 1.43 for 5 stimuli) (Geran and Travers 2006) 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. Because the entropy measure is unable to deal with negative proportions and excitatory responses were more frequent and of greater magnitude, H-values are based only on excitatory responses (Smith and Travers 1979).

Further statistical analyses were conducted using appropriate ANOVA (Statistica; StatSoft). Spontaneous firing rate prior to each stimulus was averaged for each neuron, and average baseline firing rate for each neuron group then was evaluated using a one-way ANOVA. One-way ANOVAs or independent t-tests were used for comparisons of response latencies to individual chemical stimuli between neuron groups. One-way repeated-measure (RM) ANOVAs were used to evaluate response frequency and latency to chemical stimuli within neuron groups except for NaCl generalists in which every neuron responded to only two chemical stimuli, thus comparisons were made with a paired t-test. One-way RM ANOVAs were used to calculate the breadth of tuning over time within neuron groups. Two-way RM ANOVAs were used to evaluate the effect of stimulus (4 basic taste stimuli and KCl) on average firing rates during 5 s of stimulation as well as the first and second 2-s epochs (time × taste). Acid-generalist neurons fitting the criteria for stimulus-evoked inhibition to 0.01 M citric acid during the first second were compared against all other acid generalists using a two-way RM ANOVA (type × time). Significant main effects or interactions ($P < 0.05$) of ANOVAs were further examined using Student Newman-Keuls tests except in the two-way RM ANOVA over time in which planned comparisons within and between subjects were analyzed using contrast coefficients. We used the Poisson distribution to indicate significant (1) from nonsignificant (0) responses to citric acid and KCl in all 47 neurons. A phi correlation coefficient was generated for citric acid and KCl. Graphic and Table data are presented as group means ± SE ($n \geq 3$).

RESULTS

Basic firing characteristics

We recorded responses from 47 geniculate ganglion neurons to lingual application with the four basic taste stimuli and to KCl while simultaneously recording the summated receptor potential from the anterior tongue (EGG) (see Fig. 1 for examples). For a subset of the neurons ($n = 25$), we assessed the efficacy of 1 μM benzamil HCl, a selective ENaC channel blocker on the responses to 0.1 M NaCl. Hierarchical cluster analysis grouped neurons based on similarity of responses to the basic taste stimuli as shown in the dendrogram in Fig. 2. Responses to only the four basic taste stimuli were included in the cluster analysis for comparison with those of our previous single unit investigations in the geniculate ganglion (Breza et al. 2006, 2007; Lundy and Contreras 1999). Analysis of agglomeration by way of the screen plot (data not shown) indicated that an abrupt upward deflection occurs around 0.3, separating the neurons into five groups, as indicated by the solid vertical line in the cluster analysis. The final analysis shows that there are four groups of neurons: 15 sucrose specialists, 17 NaCl specialists, 6 NaCl/QHCl generalists, and 8 acid generalists, with only 1 outlier (Qnas). This outlier was included in the acid-generalists group, as it was more similar to it than any other group (see Figs. 2 and 3).

Figure 3 shows the response profiles of all 47 neurons grouped according to the cluster analysis and arranged within group by the stimulus evoking the best response frequency over 5 s of stimulation. As can be seen, 0.5 M sucrose was an effective stimulus for ~30–35% of the neurons, mostly sucrose specialists responding between 5 and 17 spike/s and largely unresponsive to the other stimuli. Overall, 0.1 M NaCl was the most effective stimulus eliciting responses in ~70% of

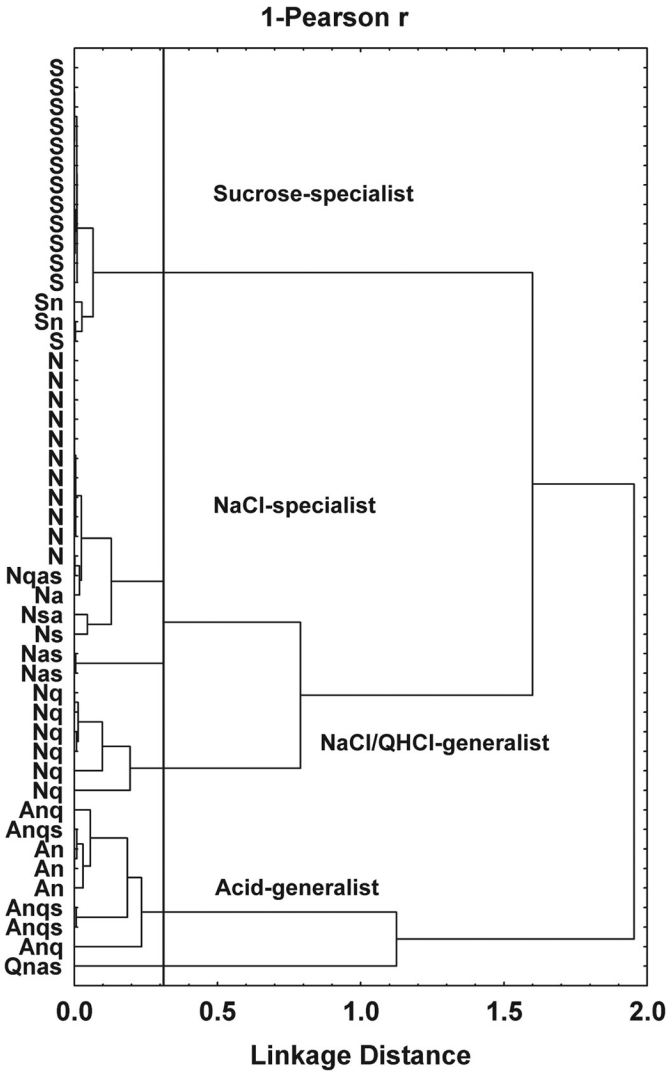

FIG. 2. Dendrogram showing the results of the hierarchical cluster analysis. Next to each neuron is the capital letter indicating the taste stimulus (S, 0.5 M sucrose; N, 0.1 NaCl; A, 0.01 M citric acid; Q, 0.02 M QHCl) that evoked the best response, followed by small letter(s) indicating taste stimuli that evoked a significant response indicated by the Poisson distribution.

the neurons from all neuron groups except sucrose specialists. Quinine was an effective stimulus for NaCl/QHCl generalists and acid generalists, while citric acid elicited responses primarily in acid generalists.

Spontaneous activity

Table 1 shows the average baseline spontaneous firing rate for each neuron group. In general, baseline firing rate tended to be higher with artificial saliva compared with the baseline rate of our previous investigations where deionized/distilled H_2O served as the rinse solution (Breza et al. 2006, 2007; Lundy and Contreras 1999). This was most evident for acid-generalist neurons as their mean spontaneous firing rate was two to three times higher than that observed in our previous investigations (Breza et al. 2006, 2007; Lundy and Contreras 1999). In fact, a one-way ANOVA revealed a significant main effect of neuron type on spontaneous firing rate [$F(3,43) = 4.00$, $P = 0.01$] and post hoc tests showed that the average spontaneous firing rate of acid generalists was significantly greater than that of each of the other neuron groups (all P values <0.05), which were not significantly different from each other.

Response latency

Figure 4 shows the percentage of neurons (by group) that responded, as determined by the Poisson distribution and their corresponding response latency to each chemical stimulus across the entire sample of 47 neurons and separately for each neuron group. As can be seen, each neuron group exhibited a unique response latency profile across the basic taste stimuli and KCl. As a rule, the preferred stimulus elicited the highest frequency response (Fig. 5) at the shortest latency (Fig. 4), with acid generalists the only exception. Specifically, the sucrose response latency of sucrose specialists was compared with that against all other neurons that responded to sucrose (Fig. 4). A one-way ANOVA revealed a significant main effect of neuron type on sucrose response latency [$F(2,21) = 12.27$, $P < 0.001$]. Post hoc analysis showed that the sucrose response latency of sucrose specialists was significantly shorter than that of NaCl specialists, which was significantly shorter than that of acid generalists (P values <0.05). Similarly, a one-way ANOVA revealed a significant main effect of neuron type on NaCl response latency [$F(2,29) = 4.28$, $P < 0.05$]. Post hoc analysis showed that the NaCl response latency of NaCl specialists and NaCl/QHCl generalists was not different from each other, but both were significantly shorter than that of acid generalists (P values <0.05). The NaCl responses of sucrose specialists were not included in the analysis because of the small number of responsive neurons (Fig. 4). Interestingly, there were no significant neuron group differences in response latency to citric acid (NaCl specialists vs. acid generalists), QHCl (NaCl/QHCl generalists vs. acid generalists), or KCl (NaCl specialists vs. acid generalists) even though there were a sufficient number of neurons that responded to each chemical stimulus within each neuron group to make accurate comparisons (Fig. 4). Specific within subject comparisons are described in the following text in sections dealing with each neuron group.

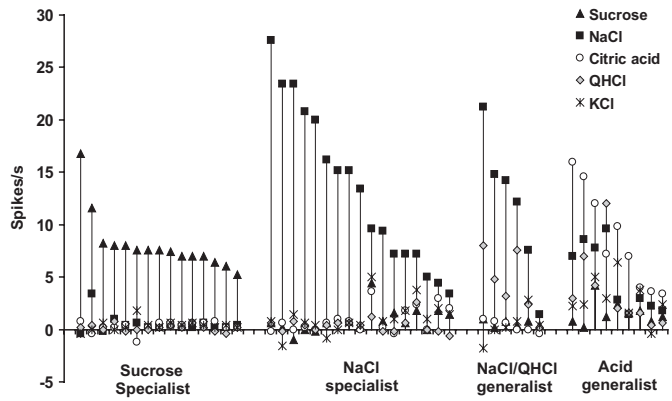

FIG. 3. Responses by each neuron to 0.5 M sucrose, 0.1 M NaCl, 0.01 M citric acid, 0.02 M QHCl, and 0.01 M KCl. Within each group, neurons were arranged by response (spike/s relative to baseline) to the best stimulus in descending order from left to right.

TABLE 1. *Baseline spontaneous firing rate for each neuron group*

Neuron Type	Present Baseline	Breza et al. (2007) Baseline
Sucrose specialists	0.75 ± 0.41	0.22 ± 0.17
NaCl specialists	1.10 ± 0.23	0.43 ± 0.10
NaCl/QHCl generalists	0.97 ± 0.23	1.58 ± 0.39
Acid generalists	2.77 ± 0.75*	0.79 ± 0.10

Baseline spontaneous firing rate (spike/s) for each neuron group after adaptation to artificial saliva in the present study and after adaptation to de-ionized water in our previous study (Breza, et al. 2007). Values are shown as means ± SE. *, significantly different from all neuron groups in the present study.

Responses to citric acid and KCl for each neuron group

All neurons responded with the highest firing rate to one of the four basic taste stimuli (see Figs. 2 and 3 for comparisons). As shown in Figs. 2–4, the majority of specialist neurons as well as NaCl/QHCl generalists were unresponsive to citric acid or KCl or in a few cases, inhibited by them. In fact, if a neuron was unresponsive to citric acid, it was unresponsive to KCl. Indeed of the five NaCl specialists that responded to citric acid, all five responded to KCl, whereas one NaCl specialist was inhibited by KCl and was unresponsive to citric acid. In contrast to all other neuron groups, every acid-generalist neuron responded to citric acid, and all but one responded robustly to KCl. Thus as a general rule, if a neuron responded to citric acid, then it responded to KCl, which is consistent with calcium imaging studies of peripheral taste cells in vitro (Huang et al. 2008; Tomchik et al. 2007). In fact, this observation is supported by a positive correlation between citric acid and KCl (phi = 0.81, $P < 0.001$).

Response patterns to the four basic taste stimuli and to KCl

SUCROSE-SPECIALIST NEURONS (N = 15). Figure 5 shows the average 5-s spontaneous activity and 5-s response frequency profile of gustatory neuron types. A two-way RM ANOVA revealed a significant main effect of stimulus [$F(4,56) = 118.31$, $P < 0.001$] and time [$F(2,28) = 3.75$, $P < 0.05$] on firing rate (Fig. 5). Post hoc analyses of stimulus showed that the sucrose response was greater than the responses to all other chemical stimuli (P values <0.001). There was also a significant interaction between stimulus and time [$F(8,112) = 5.00$, $P < 0.001$]. Post hoc tests of the interaction showed that the firing rate to sucrose was greater in the first 2 s of application compared with the average firing rate over 5 s or in the last 2 s (P values <0.01; see Fig. 5).

A one-way RM ANOVA revealed a significant effect of time on breadth of tuning [$F(2,28) = 3.52$, $P < 0.05$; see Table 2]. Post hoc analyses showed that sucrose specialists were more narrowly tuned during the first 2 s than the last 2 s of stimulation ($P < 0.05$). As shown in Fig. 4, only 2/15 sucrose-specialist neurons responded to NaCl over 5 s of stimulation based on the Poisson distribution; however, a modest but significant increase in NaCl firing rate was evident during the last 2 s of stimulation for more neurons ($n = 4$). Furthermore, the NaCl response latency of sucrose-specialist neurons was >2.4 times longer than the sucrose response latency. This delayed NaCl response of sucrose specialists contributes to the increased breadth of tuning during the last 2 s (see Table 2). Additionally, there was a tendency for a slight increase or decrease (nonsignificant via Poisson distribution) in neural activity in response to either citric acid and QHCl during the last 2 s of stimulation from 8 of 15 sucrose specialists; this ultimately increases the entropy value for those neurons during this time period. Because of the small number of neurons significantly excited or inhibited by secondary chemical stimuli, these neurons were not included in the statistics of response latency.

NaCl-SPECIALIST NEURONS (N = 17). A two-way RM ANOVA revealed a significant main effect of stimulus [$F(4,64) = 42.19$, $P < 0.001$] and time [$F(2,32) = 7.85$, $P < 0.01$] on firing rate (Fig. 5). Post hoc analyses of stimulus showed that the NaCl response was greater than the response to any other stimulus (P values <0.001). Post hoc analyses of time showed that responses to taste stimuli were less in the first 2 s compared with the average firing rates over 5 s or in the last 2 s (P values <0.05), though we are unsure if this effect was significant for a particular taste stimulus because there was no significant interaction between taste and time.

A one-way RM ANOVA revealed a significant effect of time on breadth of tuning [$F(2,32) = 13.85$, $P < 0.001$; see Table 2]. Post hoc analyses showed that NaCl specialists were more narrowly tuned during the first 2 s than the last 2 s of

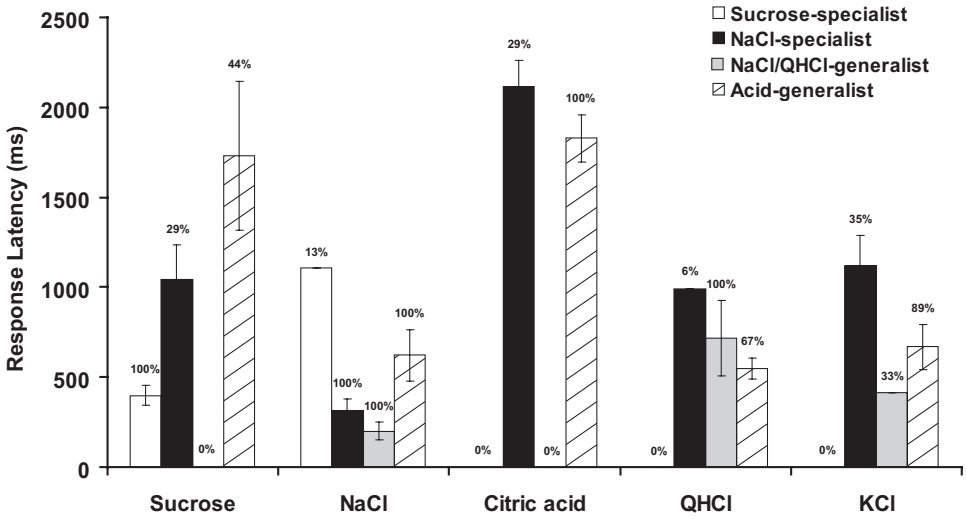

FIG. 4. Mean response latencies (milliseconds) and percentage of neurons (by group) responding (excitation or inhibition) to the 4 basic taste stimuli and to 0.1 M KCl as indicated by the Poisson distribution.

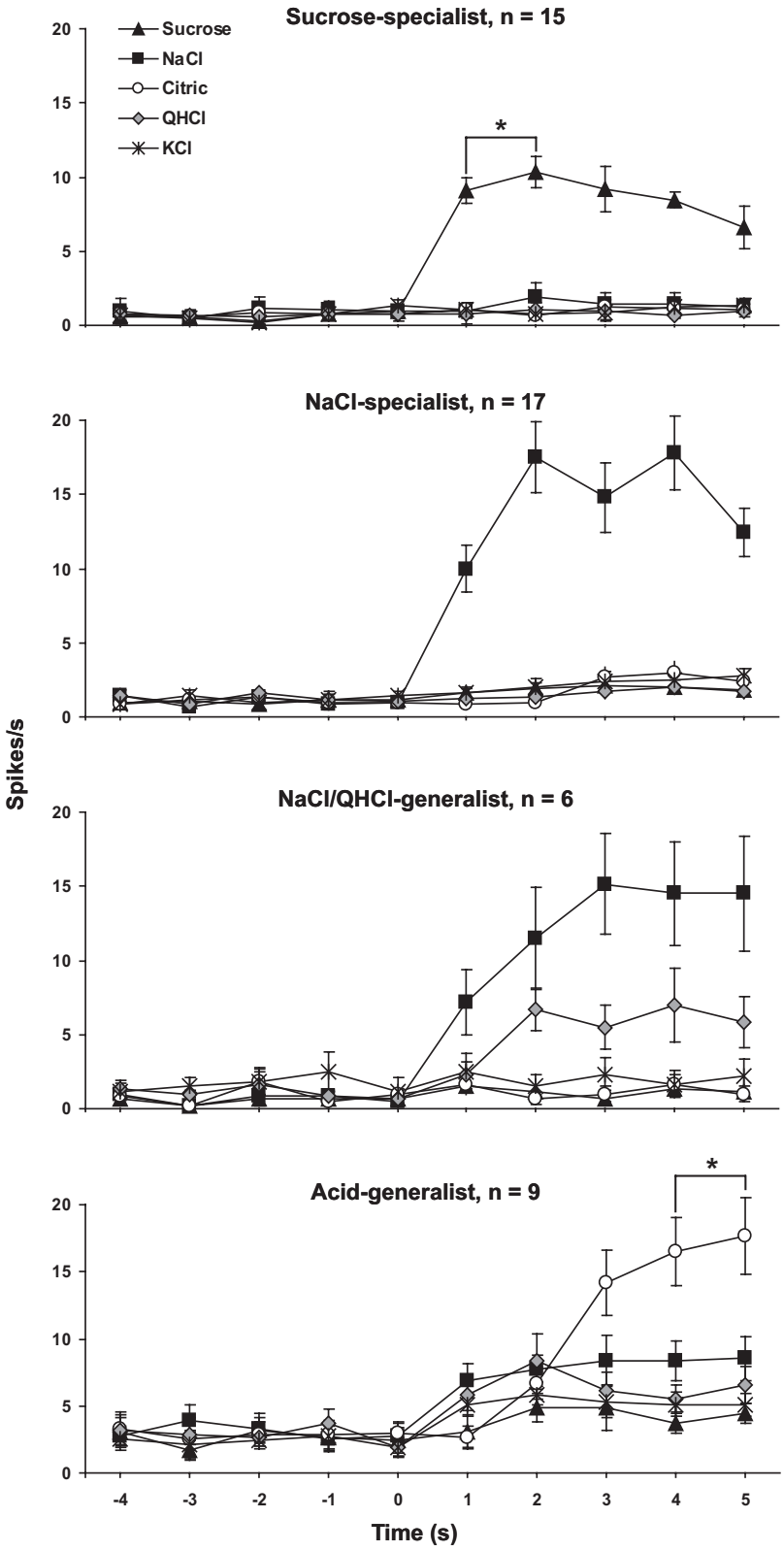

FIG. 5. Mean baseline and response frequency (spike/s) by each neuron group to the 4 basic taste stimuli and to 0.1 M KCl. *, significance ($P < 0.01$).

stimulation ($P < 0.001$) or over 5 s of stimulation ($P < 0.01$). This is in agreement with the fact that only 5 of the 17 NaCl specialists (29%) responded to sucrose and citric acid and did so only after a long delay (see Fig. 4); this also accounts for the increase in breadth of tuning during the last 2 s of stimulation (see Table 2). A one-way ANOVA performed on response latency revealed a main effect of chemical stimulus [$F(3,28) = 23.87$, $P = 0.001$]. Post hoc analyses showed that the average response latency to NaCl was significantly shorter than that to sucrose, citric acid, and KCl ($P < 0.01$).

TABLE 2. *Breadth of tuning for each neuron group*

Neuron Type	5 s	First 2 s	Last 2 s
Sucrose specialists	0.36 ± 0.03	0.31 ± 0.05*	0.44 ± 0.05
NaCl specialists	0.43 ± 0.07	0.30 ± 0.07*†	0.49 ± 0.06
NaCl/QHCl generalists	0.57 ± 0.04	0.58 ± 0.03	0.59 ± 0.08
Acid generalists	0.85 ± 0.03	0.81 ± 0.04	0.73 ± 0.04†

Breadth of tuning (basic taste stimuli and to KCl), indicated by *H* values, for each neuron group. Values are shown as means ± SE. *, significantly different from last 2 s; †, significantly different from 5 s.

The average response latency to citric acid was longer than that to sucrose and KCl (P values <0.001), which were not different from each other.

NaCl/QHCL-GENERALIST NEURONS (N = 6). A two-way RM ANOVA revealed a significant main effect of stimulus [$F(4,20) = 13.31$, $P < 0.001$] and time [$F(2,10) = 7.51$, $P = 0.01$ on average firing rate (Fig. 5). Post hoc analyses of stimulus showed that the NaCl response was greater than all other taste stimuli ($P < 0.001$) and that QHCl responses were greater than those to sucrose, citric acid, and KCl ($P < 0.05$). Post hoc analyses of time showed that the responses to all taste stimuli were less during the first 2 s of stimulation compared with the average firing rate over 5 s or during the last 2 s ($P < 0.05$). There was not a significant interaction effect between taste stimulus and time most likely because of the large error associated with the responses from the small number of neurons recorded ($n = 6$).

A one-way RM ANOVA revealed no significant effect of time on breadth of tuning (see Table 2), indicating that these neurons responded consistently to only two chemicals over time. A paired *t*-test on response latency to NaCl and QHCl revealed a significant effect ($T = -3.55$, $P = 0.01$), indicating that the response latency to NaCl was significantly shorter than that to QHCl (see Fig. 4).

ACID-GENERALIST NEURONS (N = 9). A two-way RM ANOVA revealed significant main effects of stimulus [$F(4,32) = 9.73$, $P < 0.001$] and time [$F(2,16) = 15.64$, $P < 0.001$] on firing rate (Fig. 5). Post hoc analyses showed that the citric acid response was greater than that of all other taste stimuli ($P < 0.01$). Furthermore, the response to NaCl was significantly greater than that to sucrose ($P < 0.05$) but was not different from the responses to QHCl or KCl. There was also a significant interaction between taste stimulus and time [$F(8,64) = 14.66$, $P < 0.001$]. Post hoc tests of the interaction showed that the firing rate to citric acid was greater in the last 2 s of application compared with the average firing rate over 5 s or the first 2 s ($P < 0.01$; see Fig. 5) as clearly shown in Fig. 5. In contrast, the responses to sucrose, NaCl, QHCl, and KCl did not differ across time of measurement. Interestingly, during the first 2 s of stimulation, the responses to sucrose, NaCl, citric acid, QHCl, and KCl were not significantly different from one another, indicating that these neurons cannot discriminate between taste qualities solely based on spike frequency during the period of behavioral identification (Halpern and Tapper 1971).

A one-way RM ANOVA revealed a significant effect of time on the breadth of tuning [$F(2,16) = 3.72$, $P < 0.05$; see Table 2]. Post hoc analyses showed that Acid generalists were more narrowly tuned during the last 2 s of stimulation compared with the average 5 s of stimulation ($P < 0.05$). This is in agreement with the fact that the citric acid response was much greater than that to all other stimuli during this time period (see Fig. 5). A one-way ANOVA on response latency revealed a main effect on chemical stimulus [$F(4,31) = 11.93$, $P = 0.001$]. Post hoc analyses showed that the average response latency to NaCl, QHCl, and KCl was significantly shorter than that to sucrose and citric acid ($P < 0.001$), which were not significantly different from each other (see Fig. 4).

Interestingly, we observed more cases of stimulus-evoked inhibition to citric acid than to any other taste stimulus from any of the cell types recorded. In fact, 33% of the neurons were inhibited by citric acid in the first second of stimulation as indicated by the Poisson distribution (Fig. 6). A two-way RM ANOVA of acid-generalist neurons revealed a significant effect of time on citric acid responses [$F(9,63) = 1,480$, $P < 0.001$] and a significant interaction between the type of response and time [$F(9,63) = 2.25$, $P < 0.05$]. Planned comparisons for baseline firing rate between neuron subtypes revealed that neurons fitting the criteria for stimulus-evoked inhibition had a significantly higher baseline firing rate than those that did not fit this criteria [$F(1,7) = 6.54$, $P < 0.05$]. Within the inhibitory neuron subtype, planned comparisons revealed responses to citric acid during the first second were significantly less than baseline [$F(1,7) = 11.81$, $P = 0.01$]. Interestingly, planned comparisons revealed that the response frequency of neurons from the inhibitory subtype returned to baseline values immediately after the first second and remained unchanged until the fourth [$F(1,7) = 5.81$, $P < 0.05$] and fifth second of citric acid stimulation [$F(1,7) = 7.45$, $P < 0.05$]. In contrast,

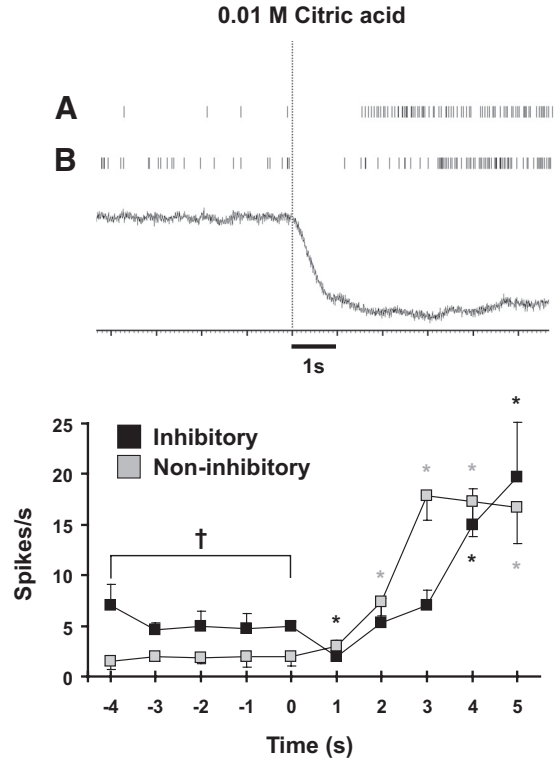

FIG. 6. Mean responses (spike/s) by inhibitory (■, $n = 3$) and noninhibitory (▒, $n = 6$) acid-generalist neurons to stimulation with 0.01 M citric acid for 5 s. * and gray star, within group significance ($P < 0.05$), corresponding to inhibitory and noninhibitory acid generalists, respectively. †, between group significance ($P < 0.05$).

planned comparisons revealed that responses to citric acid by noninhibitory acid generalists during the first second of stimulation were not different from baseline [$F(1,7) = 3.23$, $P = 0.12$]. However, unlike neurons from the inhibitory subtype, planned comparisons revealed that the response frequency increased significantly from baseline after 1 s [$F(1,7) = 14.75$, $P < 0.01$] and remained significantly different from baseline at each point thereafter [$F(1,7) = 57.01$, $P < 0.001$; $F(1,7) = 18.86$, $P < 0.01$; and $F(1,7) = 15.31$, $P < 0.01$, respectively].

NaCl concentration and benzamil

Figure 7 shows the effect of ascending NaCl concentration (0.03, 0.1, 0.3, and 0.5 M) on each neuron group. In general, increasing NaCl concentration increased response frequency and decreased response latency systematically with the response latency of NaCl/QHCl generalists the only exception. One-way RM ANOVAs revealed a significant concentration effect on response frequency [$F(3,33) = 51.32$, $P < 0.001$] and latency [$F(2,22) = 8.94$, $P = 0.001$] of NaCl-specialist neurons. Post hoc tests showed that response frequency increased systematically with increasing concentration ($P < 0.001$), reaching an asymptotic level between 0.3 and 0.5 M, whereas response latency decreased systematically with increasing concentration ($P < 0.05$).

With respect to the NaCl/QHCl generalists, one-way RM ANOVAs revealed a significant concentration effect on response frequency [$F(3,9) = 21.38$, $P < 0.001$], whereas response latency was unaffected by concentration [$F(2,8) = 0.41$, $P = 0.68$]. Post hoc analysis showed that response

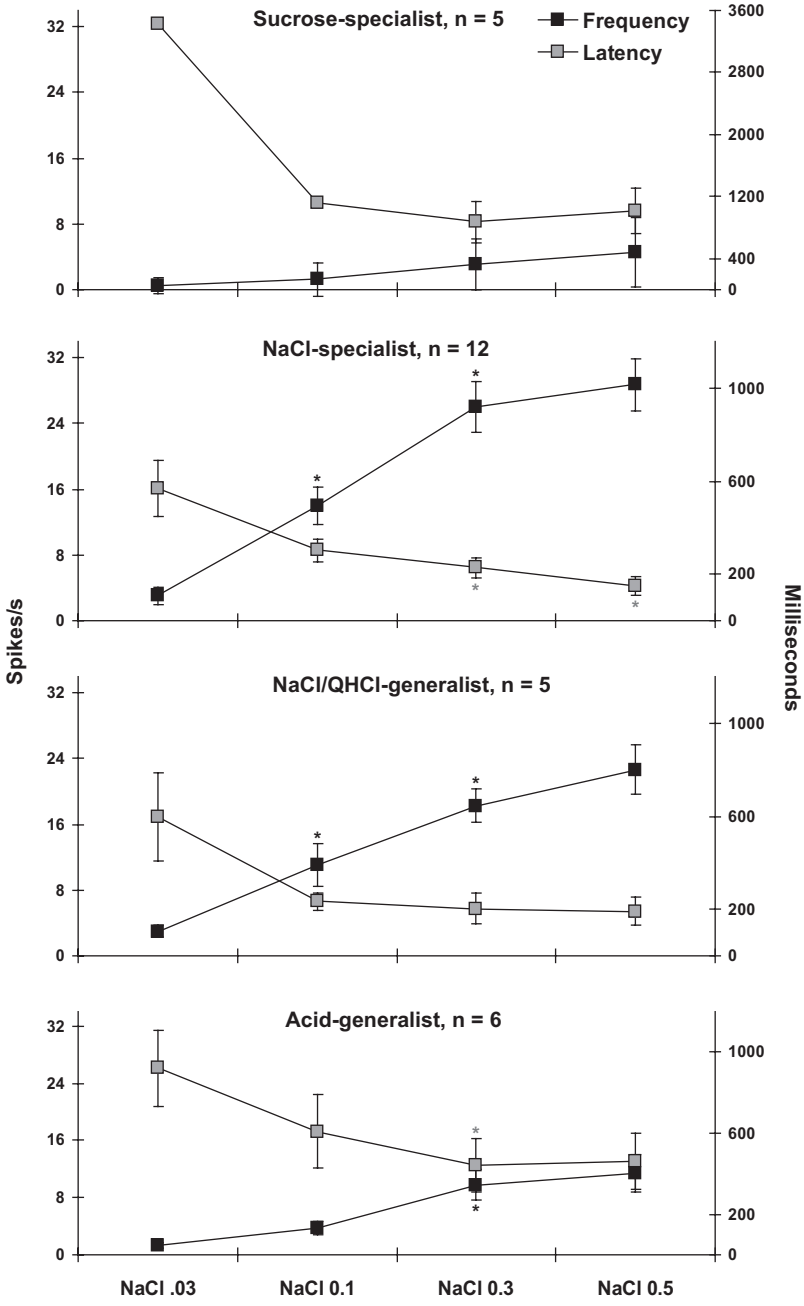

FIG. 7. Mean response frequency (spike/s relative to baseline) and response latency (milliseconds) to 4 concentrations of NaCl (0.03–0.5 M) for each neuron group. *, significant differences ($P < 0.05$) in response frequency from the adjacent lower concentration. Gray star, significant differences ($P < 0.05$) in response latency from the adjacent lower concentration.

frequency increased systematically with increasing concentration, reaching an asymptotic level between 0.3 and 0.5 M ($P < 0.05$).

With respect to acid generalists, one-way RM ANOVAs revealed a significant concentration effect on response frequency [$F(3,15) = 16.55$, $P < 0.001$] and response latency [$F(2,10) = 7.11$, $P = 0.01$]. Post hoc analysis showed that response frequency to the two highest NaCl concentrations (0.3 and 0.5) were not significantly different from one another but were significantly greater than the two lower NaCl concentrations (0.03 and 0.1 M), which also did not differ from one another (P values <0.01). Similarly, post hoc analysis showed that the response latency to 0.1 M NaCl was significantly longer than the latency to 0.3 and 0.5 M NaCl ($P < 0.05$), which did not differ from one another.

Figure 8 shows the effect of 1 μM benzamil HCl on 0.1 M NaCl responses for each neuron group. Consistent with previous investigations using amiloride (Hettinger and Frank 1990; Lundy and Contreras 1999; Ninomiya and Funakoshi 1988), benzamil decreased NaCl firing rate only in NaCl-specialist neurons. Specifically, a one-way RM ANOVA revealed a significant main effect of drug on the response frequency to 0.1 NaCl in NaCl-specialist neurons [$F(3,30) = 10.98$, $P < 0.001$]. Post hoc analysis revealed that response frequency to NaCl + benzamil was significantly lower than NaCl responses before and after benzamil ($P < 0.001$). It is worth noting that 2 of these 11 NaCl specialists were responsive to citric acid and KCl. NaCl responses in these neurons were unaffected by benzamil. Furthermore, a one-way RM ANOVA revealed a significant drug effect on response latency to 0.1 M NaCl in

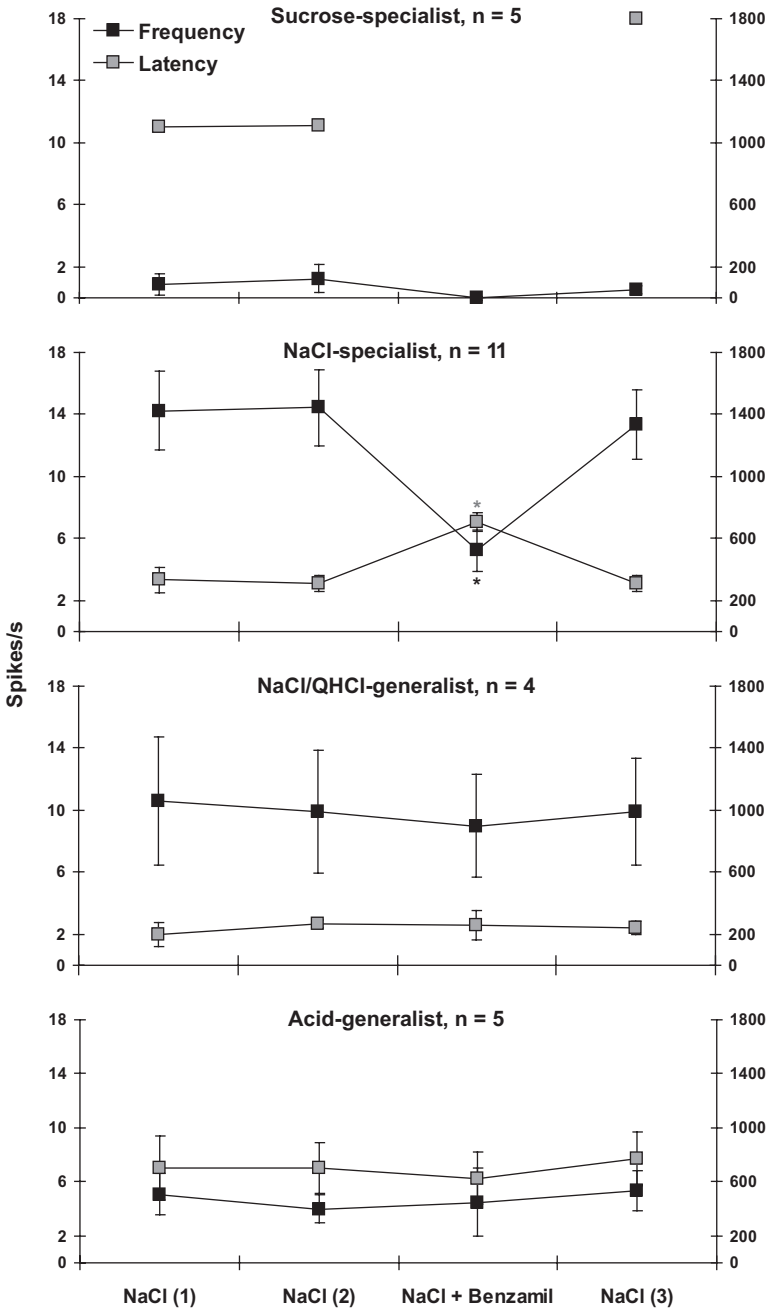

FIG. 8. Mean response frequency (spike/s relative to baseline) and response latency (milliseconds) to 0.1 M NaCl presented alone twice before and once after presentation of NaCl mixed with 1 μM benzamil for each neuron group. *, the response frequency to NaCl + benzamil is significantly different ($P < 0.001$) from 0.1 M NaCl alone. Gray star, the response latency to NaCl + benzamil is significantly different ($P < 0.01$) from 0.1 M NaCl alone.

NaCl-specialist neurons [$F(3,24) = 8.97$, $P < 0.001$]. Post hoc analysis revealed that the response latency to NaCl + benzamil was significantly longer than NaCl response latency before and after benzamil treatment ($P < 0.01$). Benzamil had no effect on response frequency and response latency of NaCl/QHCl- and acid-generalist neurons. Interestingly, 2 of 15 sucrose specialists responded significantly to 0.1 M NaCl and benzamil eliminated these responses. However, statistical analyses were not performed because of the majority of neurons were unresponsive to NaCl alone, thus making it impossible to show any effect of drug.

DISCUSSION

There are several important findings from the present work made possible, in part, by the inclusion of a reference signal (EGG) marking the time when the stimulus contacts the lingual epithelium. Foremost, spike timing, arising from response latency differences between stimuli across neurons types, seems to be an informative coding signal for discrimination of chemosensory stimuli in the rat geniculate ganglion. In general, the stimulus that elicited the highest response frequency did so at the shortest response latency by a wide margin in comparison to responses to secondary stimuli. This was especially true for narrowly tuned specialist neurons that responded robustly to only one stimulus and weakly, if at all, with long latency to secondary stimuli. This response pattern was true for all neuron types except acid-generalist neurons. Paradoxically, their response latency to secondary stimuli (NaCl, KCl, and QHCl) was shorter in contrast to a threefold higher response frequency to citric acid. In fact, acid-generalist neurons responded with a low steady firing rate to the three secondary stimuli throughout the 5-s stimulation period and robustly to citric acid beginning about 2 s after stimulus onset. Second, this difference in temporal response pattern across neuron types had a large influence on their breadth of tuning to chemical stimulation. On the one hand, sucrose- and NaCl-specialist neurons were found to be significantly more narrowly tuned during the first 2 s of stimulation when behavioral discrimination of stimulus quality and intensity occur. On the other hand, NaCl/QHCl-generalist neurons' breadth of tuning did not differ significantly between the first 2 s, last 2 s, or the entire 5-s stimulation period, whereas acid-generalist neurons' breadth of tuning was more narrowly tuned during the last 2 s. Third, we are the first to report that rat geniculate ganglion neurons respond to the four basic chemical stimuli not just with excitation but also with inhibition. This was especially true for acid-generalist neurons, the spontaneous firing rate of which was higher in a background of artificial saliva and citric acid inhibited firing rate in a significant portion of the neurons. These major findings will be discussed in greater detail in the following text with respect to how the present results compares with previously reported findings on response profiles of peripheral gustatory neurons. Additionally, we will discuss the value of using EGG and artificial saliva in the present study, and the implications the results may have to taste bud processing and peripheral coding mechanisms.

Response profiles of peripheral gustatory neurons

In the present and our previous investigations of the geniculate ganglion (Breza et al. 2006, 2007; Lundy and Contreras 1999), we used a stimulation system that delivered solution to the tongue surface at a controlled temperature (10, 15, 25, 35, or 40°C) and uninterrupted flow rate (50 μl/s) between rinse and stimulus so that the neural response record reflected solely chemical quality and intensity and was devoid of transient tactile- and thermal-evoked spikes. The present study is our first to use artificial saliva (Hirata et al. 2005), instead of de-ionized water, as the rinse solution and the solvent for all chemical stimuli in the rat. Also with the addition of the EGG, we reduced the chemical stimulation duration from 15 s in our previous studies to 5 s in the present study. With these factors in mind, there were similarities as well as some differences between the present and our earlier reported findings.

Based on the 5-s response frequency to the basic taste stimuli (in artificial saliva), the cluster analysis separated gustatory neurons nearly flawlessly into four distinct groups with unique response profiles (see Fig. 1 for examples) largely consistent with our prior observations (Breza et al. 2006, 2007; Lundy and Contreras 1999). The four groups consisted of two narrowly tuned to sucrose (sweet) and NaCl (salty), one moderately tuned to NaCl and quinine hydrochloride (bitter), and one broadly tuned to all the basic taste stimuli with a best response frequency to citric acid (sour). Consistent with previous single fiber studies using the ENaC blocker amiloride (Hettinger and Frank 1990; Lundy and Contreras 1999; Ninomiya and Funakoshi 1988), we found that 1 μM benzamil profoundly reduced NaCl responses in NaCl specialists, but not in NaCl/QHCl- and Acid-generalist neurons that were also highly responsive to NaCl stimulation. In general, the breadth of tuning across neuron types also was largely consistent with our most recent investigation (Breza et al. 2007). However, there were three major differences between the current investigation and those of a prior investigation (Breza et al. 2007), where the concentrations of the basic taste stimuli (in H_2O) and temperature were identical.

The first major difference was the present study's absence of a broadly tuned cluster of neurons that responded best to NaCl, second-best to citric acid and least to quinine; we called this cluster NaCl-generalists$_I$ neurons (Breza et al. 2007). In our prior study (Breza et al. 2007), the sample consisted of only 13% (7/52) NaCl-generalists$_I$ neurons. We suspect that a similar cluster of neurons may be somewhat evident within the present NaCl-specialist group seen in the tail end of the distribution of Figs. 2 and 3 but did not emerge statistically as a separate group in the cluster analysis. This subset of neurons within the distribution were most responsive to NaCl, weakly responsive to sucrose, citric acid, and KCl, and unresponsive to QHCl.

The second major difference was the threefold greater percentage of sucrose-specialists neurons observed in the present investigation compared with our prior studies of the rat geniculate ganglion (Breza et al. 2006, 2007; Lundy and Contreras 1999). In fact, 32% (15/47) of the present sample consisted of sucrose-specialist neurons compared with only 9% (16/175) combined from our prior ganglion studies (Breza et al. 2006, 2007; Lundy and Contreras 1999). This is particularly striking in contrast to the greater similarity in the proportion of NaCl specialists (17/47 vs. 23/52), NaCl/QHCl generalists (6/47 vs. 11/52), and acid generalists (9/47 vs. 8/52) between the present and our most recent study (Breza et al. 2007), respectively. The remarkable difference in the number of sucrose specialists may

be attributed mostly to the ionic components of artificial saliva, which has been shown to increase the magnitude of integrated whole nerve response from the rat chorda tympani to sucrose (Matsuo and Yamamoto 1990, 1992).

A third major difference was a higher spontaneous firing rate and response rate to chemical stimulation using artificial saliva compared with our previous studies using de-ionized water as the rinse solution and solvent for test stimuli (e.g., Breza et al. 2007). Indeed, the spontaneous firing rate was 2.5–4 times higher in the present study (see Table 1) for all neuron types except NaCl/QHCl generalists. The largest effect of artificial saliva was the higher spontaneous firing rate of acid generalists allowing the identification of stimulus-evoked inhibition. We suspect that removal of ions in saliva with water rinse most likely compromised the electrochemical balance of taste bud cells and thereby lowered spontaneous activity.

With respect to response rate, spontaneous rate cannot be a factor because we subtract it from response rate in all our studies. Thus we calculate response rate as a difference score from spontaneous rate with statistical significance based on the Poisson distribution. With this in mind, perhaps the most robust effect was a higher response rate to the best stimulus for sucrose- and NaCl-specialist neurons. Sucrose specialists' response to sucrose was greater by ~2 spike/s, while NaCl specialists' response was greater by ~4 spike/s. Acid generalists also had a higher response rate to citric acid by ~4 spikes but only if the last 2 s of stimulation were taken into account. These results of greater responsiveness in geniculate ganglion neurons are in concert with recording studies of the whole chorda tympani nerve showing the importance of the inorganic constituents of saliva in heightened responses to sucrose and other sweeteners (Matsuo and Yamamoto 1990, 1992). Specifically, Matusuo and Yamamoto (1990) concluded that an increase in pH from bicarbonates and an increase in sodium concentration were responsible for the increased chorda tympani response to sucrose.

Response latency

The EGG gave us an unbiased reference signal from which to anchor the spike analysis and determine response latency of single neurons from the geniculate ganglion to chemical stimulation of the tongue. The source of the EGG signal most likely comes from the summated receptor potential and the conductance/resistance properties of the ionic and nonionic chemical stimuli used in the present investigation. As shown in Fig. 1, good conducting ionic solutions resulted in an increase in conductance (positive potential, with respect to the negative polarity of the indifferent electrode), whereas nonionic solutions (sucrose, citric acid) resulted in a decrease in conductance.

In general, highly conducting solutions (NaCl and KCl) produced larger EGG signals than poorly conducting nonionic solutions (sucrose and citric acid). In fact in NaCl-specialist neurons, EGG response magnitude increased with increasing NaCl concentration; in parallel fashion, NaCl response latency decreased progressively to a low average of 150 ms as NaCl concentration increased. Conversely, benzamil reduced NaCl response frequency and increased NaCl response latency of NaCl-specialist neurons. The fact that a more intense stimulus (NaCl) elicited a quicker and bigger response is evidence of the validity of the response latency measure. Not only is the method valid but also reliable as evident by the consistency in latency measures across stimulus trials, which also varied systematically and logically between stimuli for different neuron types. This was abetted by stimulating parameters (flow rate, temperature, rinse) that were the same for all stimuli and devoid of thermal and tactile transients when switching between rinse and stimulus. Based on unsystematic observations from our lab, we know that increasing flow rate from 50 to 100 μl/s shortens by a few milliseconds response latency to all stimuli, but the overall pattern between preferred and secondary stimuli remains the same. Thus chemical response latencies are relative to the parameters used in the investigation and should not be regarded as absolute latency values for transduction, conduction velocity.

In contrast to our lowest average response latency of 150 ms from individual NaCl-specialist neurons, Faull and Halpern (1972) reported a shorter NaCl response latency from the whole chorda tympani nerve of 27, 33, and 27 ms to 100, 500, and 1,000 mM NaCl, respectively; essentially, response latency did not vary over a full log NaCl concentration range. Faull and Halpern used a gravity flow system to stimulate fungiform taste buds. Unfortunately a gravity flow system like that used by Faull and Halpern does not provide continuous flow between rinse and chemical stimulus and thus there is coincident tactile and thermal stimulation of the receptive field. Moreover, solution presentations with gravity flow systems are typically at high flow rate of ~1–2 ml/s, 20–40 times higher than the flow rate used in the present study. It is now known that the chorda tympani nerve includes neurons that are also sensitive to tactile and thermal stimulation (Finger et al. 2005; Kosar and Schwartz 1990). Thus we suspect in Faull and Halpern's study the response latency values reflect a constant tactile and thermal effect combined with a change in NaCl concentration, and not the effect of chemical stimulation alone as in the present study.

Due to variation in EGG response magnitude, we adjusted signal amplification for optimal viewing and not show a magnitude scale in Fig. 1. With the EGG as a stimulus onset marker, we demonstrated that geniculate ganglion neurons previously characterized into a small number of separate groups by their response frequency, also differed in their temporal response pattern to chemical stimulation of the tongue. In particular, we observed dramatic response latency differences between stimuli across neurons types, substantiating spike timing as an informative coding signal for discrimination of chemosensory stimuli in the rat geniculate ganglion.

As the prototypical stimulus for sweet, sucrose evoked high-frequency spike activity with a short 400-ms delay only in sucrose-specialists neurons. They were largely unresponsive to any other stimulus. Only 2/15 sucrose-specialist neurons responded weakly to NaCl at more than twice the sucrose response latency. A small percentage of NaCl-specialist and acid-generalist neurons responded to sucrose with a substantially lower response frequency and with a far longer response latency. In short, only sucrose-specialist neurons responded quickly and robustly to sucrose and no other stimulus. The evidence indicates that sucrose-specialist neurons play an important role in coding "sweet taste" information from fungiform taste buds to the brain in rats.

In contrast to sucrose, there were three neuron groups that responded consistently with a high frequency to NaCl: NaCl specialists, NaCl/QHCl generalists, and acid generalists. Each group had a unique response frequency and response latency profile that distinguished each from the other. There are several distinguishing features of NaCl-specialist neurons important from a coding perspective. Foremost, the ENaC blocker, benzamil, only suppressed the NaCl responses of NaCl-specialists neurons and no other NaCl-responsive group. Second, NaCl-specialist neurons were significantly more narrowly tuned both in terms of response frequency as well as response latency. Like sucrose specialists, all NaCl-specialist neurons responded to 0.1 M NaCl with a short latency on the order of 300 ms, and only a small subset of these neurons also responded but with low frequency to the other test stimuli with at least three times the latency. In this respect, it is notable that just a few NaCl-specialist neurons responded weakly to the other monochloride salt, KCl, again only after a long delay. As noted previously, these physiological response features of NaCl-specialist neurons match perfectly with the selective behavioral responses of rats to salt solutions under a variety of conditions (Contreras and Lundy 2000; Frank et al. 2008; Spector and Travers 2005).

As for NaCl/QHCl generalists, they are the least commonly observed group of NaCl-responsive neurons, yet they have been consistently identified by cluster analysis in the present and our two prior geniculate ganglion studies (Breza et al. 2006, 2007). NaCl/QHCl generalists respond just to two basic taste stimuli but in a completely different manner. They respond with a high response frequency to NaCl at more than twice the rate as to quinine and with more than three times shorter response latency. NaCl/QHCl-generalist neurons are relatively unresponsive to sucrose, citric acid, KCl, as well as to MSG (Breza et al. 2007). Thus even though this group of neurons responds well to both stimuli, they clearly distinguish NaCl from quinine in response frequency and temporal response pattern.

The response profile of acid generalists may be the most intriguing of all, perhaps because it was the most broadly tuned of the neuron groups and it had the most unique temporal response patterns to chemical stimulation. Furthermore as noted previously, this group had the highest spontaneous firing rate with the tongue bathed in artificial saliva. Acid generalists responded with the highest frequency response to citric acid, but this excitatory response was only evident after about a 2-s delay following stimulus application. However, for many of these neurons, a citric-acid-evoked inhibitory response preceded the excitatory response. This was most evident in those neurons with the highest spontaneous rate although apparent but difficult to verify statistically in the other neurons of this group (Fig. 6). Acid generalists responded with a similar response frequency and response latency to the three ionic solutions: NaCl, quinine HCl, and KCl. About 50% of acid generalists responded weakly to sucrose with a similar long latency as the excitatory response latency to citric acid, which was about three times longer than that to NaCl, QHCl, and KCl.

In sum, geniculate ganglion neurons responded rapidly between 150 and 400 ms to their best stimulus after lingual application of solution. This is consistent with limited behavioral data indicating that rats can respond to taste quality within 250–600 ms of licking a solution (Halpern and Tapper 1971). In this respect, we identified four neuron groups with distinct response frequency and temporal response patterns. The sweet tasting stimulus, sucrose, activates sucrose-specialist neurons and no other neuron group and vice versa; sucrose-specialist neurons respond to sucrose and no other basic taste stimulus. This specific relationship between stimulus and neuron group is comparable for salty NaCl and NaCl specialists. Like NaCl specialists, NaCl/QHCl-generalist neurons respond with a high frequency and quickly to NaCl; however, NaCl/QHCl generalists also respond to quinine, although with a distinct temporal pattern as evident by a long-latency response to quinine. Although responsive to two basic stimuli, this neuron group clearly distinguishes one stimulus from the other. Likewise, citric acid is a clearly identifiable stimulus for acid generalists. In contrast to an immediate excitatory response to NaCl, quinine, and KCl, citric acid elicits an immediate inhibitory response followed by robust excitation. Thus whether geniculate ganglion neurons are selectively or broadly responsive, the evidence indicates that they nevertheless distinguish between best stimulus and secondary stimuli based on temporal response pattern.

Peripheral coding mechanisms: taste cells to afferents

The taste bud consists of distinct cell types each uniquely expressing receptors for one of the basic taste stimuli (Chandrashekar et al. 2006; DeFazio et al. 2006; Simon et al. 2006; Tomchik et al. 2007). Of the several different cell types identified by anatomical analysis (Witt et al. 2003; Yee et al. 2001), three are known to play a role in signal transduction. A recent patch-clamp study revealed that type I cells from mouse fungiform taste buds respond to sodium solutions by an epithelial sodium channel (ENaC) mechanism and therefore may play a role in salt taste transduction (Vandenbeuch et al. 2008). Type II cells from mouse circumvallate papillae express different components of the G protein family of receptors and therefore respond directly and uniquely to sweet, bitter, or umami stimuli (Huang et al. 2008; Tomchik et al. 2007). In contrast, type III cells may be inaccessible to the chemical environment of the oral cavity and may only receive input from other cell types in the taste bud, presumably type II and type I. Inasmuch as circumvallate type III cells respond broadly to stimuli of all basic taste qualities and typically have conventional synapses with afferent fibers, they have been hypothesized to integrate chemical responses within the taste bud (DeFazio et al. 2006; Huang et al. 2008; Tomchik et al. 2007). However, deletion of type III cells seems to specifically alter acid-elicited responses (Huang et al. 2006). Regardless, type II and possibly type I cells have been referred to as receptor cells, and type III cells as presynaptic cells. The evidence suggests that there may be direct (stimulus - receptor cell - afferent fiber) and converging (stimulus - receptor cell - presynaptic cell - afferent fiber) information processing pathways within the taste bud. Furthermore, circumvallate taste bud cells can be segregated into type II receptor or type III presynaptic cells based on their response to KCl and citric acid (Huang et al. 2008; Kataoka et al. 2008; Tomchik et al. 2007). Broadly tuned presynaptic cells respond to both stimuli, whereas narrowly tuned receptor cells are unresponsive to these two stimuli.

The information on taste buds comes mostly from isolated taste cells in mouse circumvallate papilla, whereas the present data result from stimulation of rat fungiform papilla, so it may be difficult to unite the two literatures. In fact, there is at least one major difference. As shown here and many others previously (Hettinger and Frank 1990; Lundy and Contreras 1999; Ninomiya and Funakoshi 1988), there are highly specialized afferent fibers innervating fungiform papillae that respond selectively to NaCl through an ENaC-mediated transduction mechanism. While circumvallate taste bud cells have been identified that respond selectively to sweet, bitter, or umami chemical stimuli, none have been found responding selectively to NaCl (Huang et al. 2008; Tomchik et al. 2007). In fact, it has been shown that glossopharyngeal nerve afferents that supply the circumvallate and respond to NaCl do so through a mechanism independent of ENaC and referred to as amiloride-insensitive (Formaker and Hill 1991; Kitada et al. 1998). Despite this major difference, circumvallate and fungiform (Yoshida et al. 2009a,b) taste buds consist of two broad classes of taste bud cells involved in signal transduction: those that respond selectively to basic chemical stimuli deemed "receptor" cells and those that respond broadly deemed "presynaptic" cells (Huang et al. 2008; Tomchik et al. 2007). The two receptive fields differ only in chemical sensitivity. For example, fungiform taste buds have receptor cells selectively responsive to NaCl via an ENaC mechanism (Yoshida et al. 2009a) that circumvallate taste buds lack, whereas circumvallate taste buds have receptor cells selectively responsive to bitter stimuli that fungiform taste buds lack.

In the present study, 68% of the sample consisted of narrowly tuned neurons selectively responsive to sucrose (32%) or NaCl (36%) with short latency between 150 and 400 ms. In general, these neurons were relatively unresponsive to KCl and citric acid. There were no sucrose-specialist neurons that responded to these two stimuli, and only 5/17 and 6/17 NaCl-specialist neurons responded weakly at best with a long latency to citric acid and KCl, respectively. These results are consistent with prior data from isolated taste cells of circumvallate papilla suggesting that sucrose-specialist neurons may receive input from type II receptor cells. Similarly, the present data from NaCl-specialist neurons indicate that they may receive input from type I taste cells because they have been shown to respond to sodium solutions by an epithelial sodium channel (ENaC) mechanism (Vandenbeuch et al. 2008). In contrast, broadly tuned acid-generalist neurons responded to citric acid (9/9) and KCl (8/9) with long excitatory response latency between 600–1800 ms, presumably reflecting input from type III, presynaptic cells.

Unfortunately, this relatively consistent connection made between taste bud cell type and geniculate ganglion cell type falters when considering NaCl/QHCl-generalist neurons. Although only 13% of the sample, they were an intermediate group sandwiched between the two specialist groups on one side and broadly tuned acid-generalist group on the other. All six NaCl/QHCl-generalist neurons responded to both NaCl and quinine, although with significantly higher frequency and shorter latency to NaCl. This group was unresponsive to citric acid and only 2/6 neurons responded weakly to KCl with a longer latency than to NaCl but a shorter latency than to quinine. Thus NaCl/QHCl generalists cannot easily be connected with type III, presynaptic taste bud cells. They also cannot be connected with type I taste cells because benzamil failed to suppress NaCl responses of NaCl/QHCl-generalist neurons, which suggests that their sodium sensitivity comes from another mechanism, possibly transient receptor potential vanilloid 1 (TRPV1) (Lyall et al. 2004). At this juncture, there is an obvious need for more studies of isolated cells from fungiform taste buds before closing the gap between taste bud processing and coding in afferent neurons from the geniculate ganglion.

Conclusion

Recent electrophysiological studies of central chemosensory neurons indicate that temporal information characterized by spike timing plays a critical role in discriminating taste quality in the rat brain stem (Di Lorenzo et al. 2009; Hallock and Di Lorenzo 2006) and cortex (Katz et al. 2001). Investigations of taste quality coding in the peripheral nervous system have focused mostly on analysis of spike count and largely ignored temporal features of the spike record as a coding mechanism until now. In the present study, the temporal feature of interest was response latency. To quantify with precision the response latency to chemical stimulation, EGG onset was an accurate reference point marking the time when the chemical solution first contacted the lingual epithelium and from which to separate and characterize baseline activity before and response latency and spike count during stimulation. Additionally, we used a stimulus delivery system with controlled flow rate and temperature minimizing the occurrence of tactile and thermal transients in the transition between rinse and chemical stimulation. In doing so, we were able to separate four neuron groups on the basis of their unique response latency profiles. In fact, the average response latency to a preferred chemical stimulus was two to four times shorter than the latency to a secondary stimulus in narrowly tuned specialist and moderately tuned NaCl/QHCl-generalist neurons. This had a profound influence on specialist neurons' breadth of tuning over time. Furthermore, response latency decreased and response frequency increased systematically as NaCl concentration increased. This was especially true for NaCl-specialist neurons the NaCl response latency and frequency of which were increased and decreased, respectively, by the ENaC blocker, benzamil. Paradoxically, broadly tuned acid-generalist neurons responded with the highest frequency to citric acid (>5 s) but with a threefold longer latency than its responses to NaCl, QHCl, and KCl, which did not differ from one another. Intriguingly, citric acid evoked an initial inhibitory response followed by strong excitation in acid-generalist neurons with high spontaneous frequency.

Taken together, these data provide significant supporting evidence that response latency is an informative coding signal for peripheral gustatory neurons. The present results are also consistent with accumulating evidence indicating that there are two parallel processing pathways from taste buds and afferent fibers to central targets (Roper 2009; Spector and Travers 2005; Zaidi et al. 2008). One is a narrowly tuned quick-reacting pathway presumably with primary afferent neurons receiving direct input from taste bud receptor cells with the same chemical affinity. The other is a broadly tuned and slightly slower-reacting pathway with primary afferent neurons receiving converging input indirectly from diverse receptor cells via an integrating taste bud presynaptic cell (Huang et al. 2008; Roper 2009; Tomchik et al. 2007). What these two pathways serve

functionally and whether they are organized separately in the brain awaits further investigation.

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GRANTS

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REFERENCES

Boudreau JC. Rat neurophysiological taste responses to salt solutions. *Chem Senses* 8: 131–150, 1983.

Breza JM, Curtis KS, Contreras RJ. Temperature modulates taste responsiveness and stimulates gustatory neurons in the rat geniculate ganglion. *J Neurophysiol* 95: 674–685, 2006.

Breza JM, Curtis KS, Contreras RJ. Monosodium glutamate but not linoleic acid differentially activates gustatory neurons in the rat geniculate ganglion. *Chem Senses* 32: 833–846, 2007.

Chandrashekar J, Hoon MA, Ryba NJ, Zuker CS. The receptors and cells for mammalian taste. *Nature* 444: 288–294, 2006.

Clapp TR, Medler KF, Damak S, Margolskee RF, Kinnamon SC. Mouse taste cells with G protein-coupled taste receptors lack voltage-gated calcium channels and SNAP-25. *BMC Biol* 4: 7, 2006.

Contreras RJ, Lundy RF. Gustatory neuron types in the periphery: a functional perspective. *Physiol Behav* 69: 41–52, 2000.

DeFazio RA, Dvoryanchikov G, Maruyama Y, Kim JW, Pereira E, Roper SD, Chaudhari N. Separate populations of receptor cells and presynaptic cells in mouse taste buds. *J Neurosci* 26: 3971–3980, 2006.

Di Lorenzo PM, Chen JY, Victor JD. Quality time: representation of a multidimensional sensory domain through temporal coding. *J Neurosci* 29: 9227–9238, 2009.

Faull JR, Halpern BP. Taste stimuli: time course of peripheral nerve response and theoretical models. *Science* 178: 73–75, 1972.

Finger TE, Danilova V, Barrows J, Bartel DL, Vigers AJ, Stone L, Hellekant G, Kinnamon SC. ATP signaling is crucial for communication from taste buds to gustatory nerves. *Science* 310: 1495–1499, 2005.

Fishman IY. Single fiber gustatory impulses in rat and hamster. *J Cell Comp Physiol* 49: 319–344, 1957.

Formaker BK, Hill DL. Lack of amiloride sensitivity in SHR and WKY glossopharyngeal taste responses to NaCl. *Physiol Behav* 50: 765–769, 1991.

Frank ME. The classification of mammalian afferent taste fibers. *Chem Senses Flavor* 1: 53–60, 1974.

Frank ME, Contreras RJ, Hettinger TP. Nerve fibers sensitive to ionic taste stimuli in chorda tympani of the rat. *J Neurophysiol* 50: 941–960, 1983.

Frank ME, Lundy RF Jr, Contreras RJ. Cracking taste codes by tapping into sensory neuron impulse traffic. *Prog Neurobiol* 86: 245–263, 2008.

Geran LC, Travers SP. Single neurons in the nucleus of the solitary tract respond selectively to bitter taste stimuli. *J Neurophysiol* 96: 2513–2527, 2006.

Hallock RM, Di Lorenzo PM. Temporal coding in the gustatory system. *Neurosci Biobehav Rev* 30: 1145–1160, 2006.

Halpern BP, Tapper DN. Taste stimuli: quality coding time. *Science* 171: 1256–1258, 1971.

Herness S, Chen Y. Serotonin inhibits calcium-activated K^+ current in rat taste receptor cells. *Neuroreport* 8: 3257–3261, 1997.

Hettinger TP, Frank ME. Specificity of amiloride inhibition of hamster taste responses. *Brain Res* 513: 24–34, 1990.

Hirata S, Nakamura T, Ifuku H, Ogawa H. Gustatory coding in the precentral extension of area 3 in Japanese macaque monkeys; comparison with area G. *Exp Brain Res* 165: 435–446, 2005.

Huang AL, Chen X, Hoon MA, Chandrashekar J, Guo W, Trankner D, Ryba NJ, Zuker CS. The cells and logic for mammalian sour taste detection. *Nature* 442: 934–938, 2006.

Huang YA, Maruyama Y, Stimac R, Roper SD. Presynaptic (type III) cells in mouse taste buds sense sour (acid) taste. *J Physiol* 586: 2903–2912, 2008.

Kataoka S, Yang R, Ishimaru Y, Matsunami H, Sevigny J, Kinnamon JC, Finger TE. The candidate sour taste receptor, PKD2L1, is expressed by type III taste cells in the mouse. *Chem Senses* 33: 243–254, 2008.

Katz DB, Simon SA, Nicolelis MA. Dynamic and multimodal responses of gustatory cortical neurons in awake rats. *J Neurosci* 21: 4478–4489, 2001.

Kitada Y, Mitoh Y, Hill DL. Salt taste responses of the IXth nerve in Sprague-Dawley rats: lack of sensitivity to amiloride. *Physiol Behav* 63: 945–949, 1998.

Kosar E, Schwartz GJ. Effects of menthol on peripheral nerve and cortical unit responses to thermal stimulation of the oral cavity in the rat. *Brain Res* 513: 202–211, 1990.

Lundy RF Jr, Contreras RJ. Temperature and amiloride alter taste nerve responses to Na^+, K^+, and NH^{+4} salts in rats. *Brain Res* 744: 309–317, 1997.

Lundy RF Jr, Contreras RJ. Gustatory neuron types in rat geniculate ganglion. *J Neurophysiol* 82: 2970–2988, 1999.

Lyall V, Heck GL, Vinnikova AK, Ghosh S, Phan TH, Alam RI, Russell OF, Malik SA, Bigbee JW, DeSimone JA. The mammalian amiloride-insensitive non-specific salt taste receptor is a vanilloid receptor-1 variant. *J Physiol* 558: 147–159, 2004.

Matsuo R, Yamamoto T. Taste nerve responses during licking behavior in rats: importance of saliva in responses to sweeteners. *Neurosci Lett* 108: 121–126, 1990.

Matsuo R, Yamamoto T. Effects of inorganic constituents of saliva on taste responses of the rat chorda tympani nerve. *Brain Res* 583: 71–80, 1992.

Mueller KL, Hoon MA, Erlenbach I, Chandrashekar J, Zuker CS, Ryba NJ. The receptors and coding logic for bitter taste. *Nature* 434: 225–229, 2005.

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

Ogawa H, Sato M, Yamashita S. Multiple sensitivity of chordat typani fibers of the rat and hamster to gustatory and thermal stimuli. *J Physiol* 199: 223–240, 1968.

Pfaffmann C. Gustatory nerve impulses in rat, cat and rabbit. *J Neurophysiol* 18: 429–440, 1955.

Roper SD. Parallel processing in mammalian taste buds? *Physiol Behav* 97: 604–608, 2009.

Simon SA, de Araujo IE, Gutierrez R, Nicolelis MA. The neural mechanisms of gustation: a distributed processing code. *Nat Rev Neurosci* 7: 890–901, 2006.

Smith DV, Travers JB. A metric for the breadth of tuning of gustatory neurons. *Chem Senses Flav* 4: 215–229, 1979.

Smith JC, Davis JD, O'Keefe GB. Lack of an order effect in brief contact taste tests with closely spaced test trials. *Physiol Behav* 52: 1107–1111, 1992.

Sollars SI, Hill DL. In vivo recordings from rat geniculate ganglia: taste response properties of individual greater superficial petrosal and chorda tympani neurons. *J Physiol* 564: 877–893, 2005.

Spector AC, Travers SP. The representation of taste quality in the mammalian nervous system. *Behav Cogn Neurosci Rev* 4: 143–191, 2005.

Tomchik SM, Berg S, Kim JW, Chaudhari N, Roper SD. Breadth of tuning and taste coding in mammalian taste buds. *J Neurosci* 27: 10840–10848, 2007.

Treesukosol Y, Lyall V, Heck GL, DeSimone JA, Spector AC. A psychophysical and electrophysiological analysis of salt taste in Trpv1 null mice. *Am J Physiol Regul Integr Comp Physiol* 292: R1799–1809, 2007.

Vandenbeuch A, Clapp TR, Kinnamon SC. Amiloride-sensitive channels in type I fungiform taste cells in mouse. *BMC Neurosci* 9: 1, 2008.

Witt M, Reutter K, Miller IJ. Morphology of the peripheral taste system. In: *Handbook of Olfaction and Gustation*, edited by Doty RL. Marcel Dekker, NY: 2003, p. 651–677.

Yee CL, Yang R, Bottger B, Finger TE, Kinnamon JC. "Type III" cells of rat taste buds: immunohistochemical and ultrastructural studies of neuron-specific enolase, protein gene product 9.5, and serotonin. *J Comp Neurol* 440: 97–108, 2001.

Yoshida R, Horio N, Murata Y, Yasumatsu K, Shigemura N, Ninomiya Y. NaCl responsive taste cells in the mouse fungiform taste buds. *Neuroscience* 159: 795–803, 2009a.

Yoshida R, Miyauchi A, Yasuo T, Jyotaki M, Murata Y, Yasumatsu K, Shigemura N, Yanagawa Y, Obata K, Ueno H, Margolskee RF, Ninomiya Y. Discrimination of taste qualities among mouse fungiform taste bud cells. *J Physiol* 587: 4425–4439, 2009b.

Zaidi FN, Todd K, Enquist L, Whitehead MC. Types of taste circuits synaptically linked to a few geniculate ganglion neurons. *J Comp Neurol* 511: 753–772, 2008.