INTRODUCTION

A large body of electrophysiological studies from single taste-responsive axons have been conducted to determine the peripheral nervous system’s strategy for coding taste quality (Cohen et al. 1955; Erickson et al. 1965; Fishman 1957; Frank 1974, 1991; Frank et al. 1983, 1988; Hellekant et al. 1997a,b; Hettinger and Frank 1990; Ninomiya et al. 1982; Ogawa et al. 1968; Pfaffmann 1955; Yamamoto and Kawamura 1978; Zotterman 1935). Some studies have shown that afferent fibers can be meaningfully divided into groups on the basis of their sensitivity to different classes of chemical stimuli (Boudreau et al. 1983; Frank 1974, 1991; Frank et al. 1983, 1988). When one determines the stimulus class that evokes the greatest discharge rate, the sensitivity to other chemicals can be predicted. Specifically, these prior studies indicate four types of response profiles termed S-, N-, H-, and Q-units. The S- and N-units are the most chemically selective responding best to sweet-tasting stimuli and Na+/Li+ salts, respectively. These neural elements appear to be only weakly responsive to other classes of chemical stimuli. In contrast, the H-units respond best to acids but also respond well to salts and bitter. Also broadly tuned, the Q-units respond best to bitter and are sensitive to salts and acids. An unsettled issue is the functional role that these physiological types play in discriminatory behavior.

Two primary theories have emerged to explain the chemical sensitivity of single neural elements in terms of coding information about taste quality, an across-fiber and a labeled-line theory (Erickson 1968). Briefly, the former theory emphasizes that individual neurons respond to many different compounds, and quality coding is accomplished through the differential activation across the neuron population. The latter theory, on the other hand, is critically dependent on the existence of neuron types and assumes that a particular type carries information about only one taste quality. Whether the gustatory system utilizes a population code or a labeled-line code is unresolved because either theory can adequately explain the manner in which information is used to code taste quality. Clearly, a better understanding of the stimulus sensitivity of individual taste neurons will provide insight into quality coding mechanisms.

For several years our interest has focused on salt taste coding to gain insight into the mechanisms that govern salt intake in rodents (Contreras 1989). The rat is the predominant animal model for research on the physiology of salt ingestion (Contreras 1989). In addition, the mammalian chorda tympani nerve is by far the most responsive to salt stimulation than any of the other nerves innervating taste receptor cells. Much of the current knowledge of neuron types comes largely from electrophysiological studies of the chorda tympani nerve in rats (Boudreau et al. 1983; Frank et al. 1983; Ninomiya and Funakoshi 1988) and hamsters (Frank 1973; Hettinger and Frank 1990). However, few studies of the rat chorda tympani have included stimuli other than sucrose, NaCl, HCl, and quinine. This may be due partly to the fact that most electrophysiolog-
Physical studies were from severed axons that can only be examined over a relatively short duration because of their limited viability. There is just one research group that has investigated single-cell responses of the mammalian chorda tympani by extracellular recording from intact cell somata in the geniculate ganglion (Boudreau and Alev 1973; Boudreau and White 1978; Boudreau et al. 1982, 1983). The only study conducted in rats used a large array of stimuli, but only a single concentration of each (Boudreau et al. 1983). The difficulty of exposing the geniculate ganglion, hidden within the complex bony structure of the tympanic cavity while simultaneously stimulating taste receptors probably accounts for the absence of studies using this approach. Despite the technical difficulty, the ganglion cell preparation is invaluable for obtaining stable recordings over long periods of time. We used this preparation to characterize the single-cell responses of the rat geniculate ganglion in four important ways.

First, we recorded ganglion cell responses to a single concentration of sucrose, NaCl, HCl, and quinine HCl. These stimuli represent the taste qualities of sweet, salt, sour, and bitter, respectively. The particular stimulus concentrations have been used effectively to segregate neurons into distinct groups based on the stimulus evoking the best response and the relative responses to the other stimuli (Frank 1973, 1974, 1991; Frank et al. 1983; Pfaffmann 1974). This first step was critical considering the absence of studies of the geniculate ganglion and the necessity of relating our research to the broader existing literature on single fiber recording.

Second, we examined the responses of geniculate ganglion neurons to two concentrations each of NaCl, KCl, and NH4Cl. This was done to determine how the various neuron groups of the salt-sensitive chorda tympani nerve responds to these distinguishable monochloride salts to gain insight into salt taste coding mechanisms. Little is known about coding mechanisms for salt beyond NaCl. Erickson et al. (1965) showed that NaCl evoked a pattern of activity in chorda tympani fibers that differed from that evoked by KCl and NH4Cl, which were highly correlated with each other. In the one study of the rat geniculate ganglion, Boudreau et al. (1983) examined single neuron responses just to 0.05 M NaCl, LiCl, NH4Cl, KCl, and MgCl. The results suggested that salt- and acid-sensitive neurons responded differently to the five salt stimuli. Salt-sensitive neurons responded well to NaCl and LiCl, and little if at all to the other three salts. Acid-sensitive neurons responded to all five salts, but most to NH4Cl.

Third, we used three pharmacological antagonists of epithelial ion transport to explore salt transduction mechanisms that may be associated with the different neuron groups of the chorda tympani nerve. There is considerable evidence that amiloride-sensitive sodium channels play a significant role in taste transduction for sodium salts (Gilbertson 1998). There are two studies that suggested that potassium channels blocked by 4-aminopyridine (4-AP) may play a role in taste transduction for KCl (Kim and Mistrretta 1993; Simon et al. 1988) and for NH4Cl (Kim and Mistrretta 1993). Recently, Lundy et al. (1997) found that the Na+/H+ exchanger antagonist, 5-(N,N-dimethyl)-amiloride (DMA), suppressed NH4Cl responses, indicating that Na+/H+ exchangers may be involved in NH4Cl taste transduction in rats. There have been only two studies that have investigated the influence of amiloride on single-cell responses of the chorda tympani nerve. Amiloride suppressed NaCl responses of sodium-responsive taste neurons in hamster (Ninomiya and Funakoshi 1988) and rat (Hettinger and Frank 1990). So far there have been no studies that have examined the influence of the Na+/H+ exchange antagonist, DMA, or the K+ channel antagonist, 4-AP, on single-cell responses of the chorda tympani nerve.

Finally, we controlled adapting and stimulus temperature to study geniculate ganglion responses to cooling and the influence of temperature on responses to the standard taste stimuli. The responses of the chorda tympani nerve have been shown to depend on the temperature of the stimulating solution in rats, hamsters, cats, and dogs (Fishman 1957; Lundy and Contreras 1997; Nakamura and Kurihara 1991; Ogawa et al. 1968; Yamashita and Sato 1965; Yamashita et al. 1964, 1970). For example, Yamashita and Sato (1965) found that the whole nerve responses of the rat chorda tympani to sucrose, HCl, quinine HCl, NaCl, and KCl were maximal at 30–35°C and decreased outside this range. Lundy and Contreras (1997) found that the whole nerve response to NH4Cl was much less, that to NaCl was somewhat less, and that to KCl was similar at 25°C compared with 35°C.

At a single cell level, Fishman (1957) showed that chorda tympani responses to NaCl in some neurons decreased with a decrease in temperature. Ogawa et al. (1968) and Yamashita et al. (1970) demonstrated that many single fibers in the chorda tympani responded to both chemical and thermal stimuli. Furthermore, Ogawa et al. (1968) found that sucrose sensitivity correlated positively with warming, and HCl and quinine sensitivity with cooling. Although it is clear that temperature interacts with taste, little is understood regarding thermal sensitivity and taste-temperature interaction with respect to neuron group.

In the present research, we were able to record highly consistent and stable extracellular responses from single neurons of the rat geniculate ganglion for 1.5–2.5 h, and in many instances could have been held much longer. This was done to characterize the chemical and thermal sensitivity of the rat geniculate ganglion to lingual stimulation, and to examine the effects of pharmacological antagonists of epithelial ion transport on salt transduction mechanisms. In so doing, the present results provide unequivocal confirmation of chorda tympani neuron types with a broader and clearer definition of their unique response characteristics than was previously elucidated from single-fiber studies. Additionally, the systematic characterization of chemical, pharmacological, and thermal sensitivities has uncovered a fifth physiological group of chorda tympani neurons that was not considered a discrete group previously. Our results will be discussed in terms of prior single axon studies and the role neuron types may play in coding taste quality.

**EXPERIMENT 1**

**METHODS**

**Subjects**

Extracellular recordings were obtained from the geniculate ganglion of 41 adult male (n = 25) and female (n = 16) rats weighing 250–450 g [Sprague-Dawley, CrL:CD (SD) BR, Charles River Breeding Laboratories]. Rats were housed in transparent plastic cages, a maximum of two per cage, in a temperature-controlled colony room.
on a 12–12 h light-dark cycle with lights on at 0500 h. All animals had free access to Purina Rat Chow 5001 and deionized-distilled water ad libitum.

Surgery and recording

Rats were anesthetized with urethan (1.5 g/kg body wt) and secured in a stereotaxic instrument with nonpuncture ear bars (45° taper). Supplementary doses of urethan were administered as necessary to maintain a deep level of anesthesia. Rectal temperature was monitored throughout the experiment and maintained at 36–38°C through a feedback loop connected to a heating pad. The geniculate ganglion was exposed using a dorsal approach following procedures described previously in monkeys (Beckstead and Norgren 1979) and cats (Boudreau and Alev 1973). Briefly, the occipital portion of the skull was exposed with a midline incision allowing visualization of bregma and lambda cranial sutures. A portion of the cranial lateral to the midline suture, rostral to lambda and caudal to bregma was removed. Partial aspiration of the cortical tissue allowed access to the petrous ridge of the temporal bone. Additional drilling into the petrous bone with a No. 2 surgical burr was required to expose the ganglion.

Glass-insulated tungsten electrodes (resistance between 2 and 6 MΩ) were lowered into the ganglion using a stereotaxic micromanipulator equipped with a KOPF micropositioner. Neural activity was differentially amplified (×10,000) with respect to an indifferent electrode attached to the skin overlaying the posterior cranium. Neural responses were monitored with an oscilloscope and audiometers and stored on a Vetter videocassette recorder for off-line analysis. Cambridge Electronic Design’s Spike2 hardware and software was used to convert the recorded data to digital format.

Eight experiments contained data with two neurons present in the recording, whereas one experiment had three neurons present in the recording. In general, Spike2 functions as a window discriminator in conjunction with waveform matching. The analogue signal was stored on the computer as a list of numbers that represent the waveform amplitude at equally spaced time intervals. The software was allowed to sample a small portion of the recorded data to estimate the noise and the peak-to-peak range of the data. Spike templates were formed during this initial sampling period. The amplitude of the spike crossing a trigger level (positive or negative) will detect a neural spike. Spikes will only be included in a template if more than a user defined percentage of the points in a spike fall within the template. The present study characterized a spike with 75 points and defined the percentage of corresponding points between a spike and a potential template to be >60 and the difference in amplitudes to be <10%. The software program classified subsequent spike shapes based on these parameters. Thus individual neurons in the multiunit preparations were identified based not only on spike amplitude but also on waveform similarity.

Stimulus delivery and protocols

The tongue was gently extended out from the oral cavity and fixed in place by attaching a small suture to the ventral surface of the tongue and securing the loose end of the suture to the tabletop with tape. For stimulus presentation we used a computer-controlled delivery system designed and built at Florida State University. Stimuli were presented to the anterior portion of the tongue by computer-controlled stepping motors to maintain a constant flow rate of 50 μl/s, which is considerably slower than that used by other investigators. This slower flow rate approximates the fluid volume consumed by a normal rat licking from a drinking spout obtaining ~5–7 μl/llick at a rate of 6–7 licks/s (Smith et al. 1992). By way of four independently controlled input valves, the stimulus and rinse input lines were linked with a stimulus outflow tube directed over the anterior surface of the tongue. The four input valves were controlled by a custom computer program that permitted rapid switching and/or mixing between two stimulus and two rinse channels while maintaining a continuous solution flow through the outflow tube. Therefore defining a mixing ratio between a stimulus-input line and a rinse line could dilute a stock stimulus concentration. The time required for a stimulus or water rinse to flow from an input valve to the tongue surface was ~2 s. A Peltier heat exchange device placed near the end of the stimulus outflow tube could hold the temperature of the rinse and stimulating solvents constant within a range of 5–50°C or change it within this range at a maximum of 1°C/s. A suction tube placed underneath the tip of the rat’s tongue removed solutions that flowed off the tongue.

During the recording session, deionized-distilled water flowed continuously over the tongue at 35°C before and after stimulus presentation at the same temperature unless otherwise noted. To classify neuronal types, the tongue was stimulated with 0.5 M sucrose, 0.1 M NaCl, 0.01 M HCl, and 0.02 M QHCl. Additional stimuli tested in some neurons included 0.075 and 0.3 M concentrations of NaCl, KCl, and NH₄Cl. In these instances, responses to the four standard stimuli were obtained before and after salt stimulation. Each stimulus was presented for 15 s and followed by a 90- to 150-s water rinse.

Data analysis

The difference between each neuron’s 15-s average response to a test stimulus and its 5-s average baseline rate immediately before stimulation was calculated. These measures were used in the cluster analysis, which utilized Pearson’s product-moment correlation coefficients and the average-linking method between subjects (SPSS for windows). This analysis was used to categorize individual neurons with similar response characteristics. The response rates were also used to calculate the breadth of responsiveness (H) for each neuron. H was calculated according to the formula $H = -K \sum p_l \log p_l$, where $K$ is a scaling constant (1.66 for 4 stimuli) and $p_l$ is the proportion of the response to each of the stimuli against the total response to all the stimuli (Smith and Travers 1979).

One- and two-way ANOVAs were performed to detect significant differences between groups. In some instances, post hoc contrast analyses (least significant difference method) were used to determine the source of statistically significant differences. In all experiments, data analyses were done using SPSS, and the significance of a response during stimulation was based on the Poisson distribution. P values <0.05 were considered significant. The results are shown as the means ± SE.

RESULTS

Seventy-three gustatory neurons were isolated from the geniculate ganglion and tested with each of four standard stimuli. The mean spontaneous activity of our sample was 0.37 ± 0.26 (SD) spikes/s and the average corrected response rate was 1.8 ± 3.2 spikes/s for 0.5 M sucrose, 8.5 ± 5.8 for 0.1 M NaCl, 7.3 ± 7.2 for 0.01 M HCl, and 3.5 ± 3.8 for 0.02 M QHCl. The percentage of neurons that responded to sucrose, NaCl, HCl, and QHCl was 38, 94, 68, and 61%, respectively. The duration of an individual spike (electrical representation of an action potential) was 1.02 ± 0.08 ms.

Neuronal characterization: hierarchical cluster analysis

Pearson product-moment correlation coefficients were generated between each possible pair of responses to the 4 standard stimuli in the 73 neurons. The resulting matrix of the relative similarity among the neurons was subjected to a cluster analysis to reveal any natural grouping of the data. The level at
which two neurons or groups of neurons connect refers to their overall correlation. In the dendrogram of Fig. 1, numbers near 0 refer to neurons or groups of neurons with a high degree of similarity, whereas larger numbers refer to those with a lower degree of similarity. Next to each neuron is the symbol of the stimulus that evoked the largest discharge followed by any other stimulus that evoked a response >40% of that maximum.

The cluster analysis indicates three main groups of neurons, which are separated nearly perfectly into best-stimulus categories. In the cluster labeled N, 30 of the 32 neurons responded best to NaCl and the other 2 responded best to QHCl. In the H cluster, all 33 neurons responded best to HCl, and in the S cluster, all 8 neurons responded best to sucrose. Within the N cluster, three subclusters can be identified, one composed of NaCl-specialist neurons (N1), another of NaCl-generalist neurons (N2), and another containing QHCl-generalist neurons and one NaCl-generalist neuron (Q). One NaCl-generalist neuron fell outside the N2 cluster, but it was still more closely associated with this group than any other group. Using this arrangement of neurons allows for the best-stimulus designation and relative responsiveness of each neuron to be identified throughout the study. As will be shown, these groups of neurons were associated with unique response characteristics to qualitatively different taste stimuli.

On the basis of the cluster analysis, neurons were classified as follows: 8 sucrose-specialists (11%), 18 NaCl-specialists (25%), 11 NaCl-generalists (15%), 33 HCl-generalists (45%), and 3 QHCl-generalists (4%). The specialist neurons responded above the baseline rate, nearly without exception, to one stimulus and the generalist neurons responded to at least three of the stimuli. Shown in Fig. 2, A–D, are examples of four of the five neuron classes sampled in this study. The sucrose- and NaCl-specialist neurons (Fig. 2, A and B) were narrowly tuned to sucrose and NaCl, respectively, whereas NaCl, HCl, and QHCl evoked a response, albeit differentially, in NaCl- and HCl-generalist neurons.

The response profiles of the 73 ganglion neurons to the standard stimuli are shown in Fig. 3. From left to right, these neurons were arranged on the basis of their grouping in the cluster analysis. Within each group, neurons were arranged by their response rate to the best stimulus in descending order. **Neurons 1–8** were sucrose-specialists, **9–26** were NaCl-specialists, **27–37** were NaCl-generalists, **38–70** were HCl-generalists, and **71–73** were QHCl-generalists. Examination of sucrose-specialist neurons shows that 0.1 M NaCl activated 4 of the 8 neurons, whereas 0.01 M HCl and 0.02 M QHCl evoked a significant response in only 1 neuron. In the case of NaCl-specialist neurons, it can be seen that 2 of the 18 were weakly responsive to HCl and QHCl, whereas only one neuron responded above baseline rate to 0.5 M sucrose. The generalist neurons are perceived by the cluster analysis to be different based on their relative responsiveness to NaCl, HCl, and QHCl.

The average response rates to each of the four standard stimuli (n = 73) and the two concentrations of NaCl, KCl, and NH4Cl (n = 66) are shown in Fig. 4. Among the standard stimuli, separate one-way ANOVA tests revealed a significant main effect for stimulus in each group (P values <0.01) except the QHCl-generalists (P = 0.08). This lack of a statistically significant best stimulus for the QHCl-generalists was due to the fact that the cluster analysis grouped one neuron that

**FIG. 1.** Shown is a dendrogram depicting the relative response similarity among the neurons. In the body of the dendrogram, the notations N1, N2, Q, H, and S correspond respectively to NaCl-specialist, NaCl-generalist, QHCl-generalist, HCl-generalist, and sucrose-specialist neurons. Next to each neuron is the symbol of the stimulus (S, 0.5 M sucrose; N, 0.1 M NaCl; H, 0.01 M HCl; and Q, 0.02 M QHCl) that evoked the largest discharge followed by any other stimulus that evoked a response >40% of the maximum.
responded best to NaCl with the QHCl-generalist group. The order of stimulus effectiveness for sucrose-specialists was $S > N = H = Q$. Among the three salts, the sucrose-specialist neurons responded little if at all to these chemicals. A two-way ANOVA did not reveal a main effect for stimulus, molarity, or a stimulus $X$ molarity interaction ($P$ values $>0.27$). However, the response evoked by 0.3 M NaCl was statistically different from 0 ($t = 3.5, P = 0.01$). This strong salt concentration stimulated all of the sucrose-specialist neurons above the baseline rate.

The sensitivity of NaCl-specialist neurons was quite specific to the Na$^+$ moiety. On average, the responses elicited by both concentrations of KCl and NH$_4$Cl were statistically different from 0 ($P$ values $<0.01$), but were 8–10 times lower than the responses to the same concentrations of NaCl. The response rate of this neural group also increased with an increase in NaCl concentration ($F_{1,90} = 6.0, P < 0.01$). Unlike their specialist counterparts, the NaCl-generalists responded well to HCl and QHCl ($N > H = Q > S$), whereas the responses to KCl and NH$_4$Cl equaled the rates to NaCl ($F_{2,60} = 1.1, P = 0.31$). The response rates of the NaCl-generalists did not increase significantly with an increase in stimulus concentration up to 0.3 M ($F_{1,60} = 4.0, P = 0.05$).

The QHCl-generalists may be true generalists in the sense that they appeared to respond equally to each stimulus with the exception of sucrose. Like NaCl-generalists, these neurons were equally sensitive to our range of salt concentrations. In contrast to the other two generalist types, the order of stimulus effectiveness among the four standards in HCl-generalists was $H > N > Q > S$. This neuron group was also stimulated differentially by NaCl, KCl, and NH$_4$Cl ($F_{2,192} = 31.5, P < 0.01$) and were quite sensitive to an increase in salt concentration ($F_{1,19} = 45.0, P < 0.01$). A nonsignificant interaction between stimulus and molarity indicated that the response rates differed for both concentrations of the salts ($F_{2,192} = 0.52, P = 0.59$). Post hoc analysis showed that the response rate order for HCl-generalists was NH$_4$Cl $>$ NaCl $>$ KCl.

The temporal response rates of NaCl- and HCl-generalist neurons to HCl, 0.075 M NH$_4$Cl, and 0.3 M NH$_4$Cl are shown in Fig. 5, A–C. Seconds 1–5 and 21–25 were the periods of 35°C water application, and seconds 6–20 the period of stimulus application. A greater overall sensitivity to HCl and NH$_4$Cl in the HCl-generalists is clear. On average, the response rates were greater by 6.6 spikes/s for 0.01 M HCl ($F_{1,43} = 10.6, P < 0.01$), 3.4 spikes/s for 0.075 M NH$_4$Cl ($F_{1,41} = 3.45, P = 0.07$), and 7.1 spikes/s for 0.3 M NH$_4$Cl ($F_{1,41} = 4.7, P = 0.03$). Moreover, a considerable difference between these two neuron categories was apparent in the temporal features of the response elicited by HCl (Fig. 5A). In HCl-generalist neurons, the response to 0.01 M HCl peaked early within the first second and then declined rapidly over the next two seconds followed by a steady increase. This unique temporal response pattern has been observed previously (Frank et al. 1983).

The spontaneous rate of the two specialists groups was

![Fig. 2. Examples of 4 of the 5 neuron classes sampled in this study. In the graphs labeled A–D are shown, respectively, a sucrose-specialist, a NaCl-specialist, a HCl-generalist, and a NaCl-generalist neuron. Each neuron was tested for its sensitivity to 4 chemicals: 0.5 M sucrose, 0.1 M NaCl, 0.01 M HCl, and 0.02 M QHCl. The solid black bar below each spike record represents the duration of stimulus application (15 s).](http://jn.physiology.org/10.1152/jn.00786.2017)
similar but significantly lower than the spontaneous rate of NaCl- and HCl-generalist neurons (post hoc test; P values <0.01). Spike duration was comparable among the neural groups. It should be noted that in our sample, 46% of the neurons came from females (6/8 sucrose-specialists; 8/18 NaCl-specialists; 6/11 NaCl-generalists; 11/33 HCl-generalists). However, a main effect for sex or an interaction with the stimulus variable were not evident for any neuronal category (P values >0.09). The only difference observed was in spontaneous activity in NaCl- and HCl-generalist neurons. The rate was significantly greater in males (NaCl-generalists, 1.0 spikes/s; HCl-generalists, 0.7 spikes/s) than in females (0.3 and 0.4 spikes/s, respectively). For both sexes, response rate was unrelated to spontaneous rate.

The breadth of responsiveness (entropy) for each class of neurons was also quantified, and the distribution is shown in Fig. 6. The entropy measure can range from 0 to 1 where a value of 0 corresponds to neurons that were activated by only one stimulus, and a value of 1 corresponds to neurons that were equally activated by all the stimuli. Two distributions are apparent in this figure. Every neuron with an entropy measure ≤0.4 was either a sucrose- or NaCl-specialist neuron, whereas 48 of the 54 neurons with an entropy measure >0.45 were generalist neurons. Given our stimulus array, it seems apparent that chorda tympani neurons come in two basic flavors, narrowly and broadly responsive neurons.

**EXPERIMENT 2**

In experiment 1, five groups of neurons were identified based on similarity of response profiles. One aspect of experiment 2 was to examine the relationships between salt-responsive neurons and the effects of the salt transduction antagonists amiloride, DMA, and 4-AP. In addition, the sensitivity to cooling the tongue and the effects of adaptation temperature on neural responses to the four standard stimuli were assessed in an attempt to characterize further differences between neuron types.

**METHODS**

**Stimulation protocols: salt transduction antagonists**

The ability of amiloride, DMA, and 4-AP to inhibit responses to 0.075 and 0.3 M concentrations of NaCl, KCl, and NH₄Cl was assessed in 66 of the 73 neurons classified into neuron groups in experiment 1. The tongue was stimulated with a 75% and 0% dilution of stock (0.3 M) NaCl, KCl, and NH₄Cl solution mixed with and without 1 μM amiloride and DMA as well as 5 mM 4-AP. These dilutions correspond to 0.075 and 0.3 M salt concentrations, respectively.

One micromolar amiloride and DMA were chosen based on the specificity of their sites of action at this concentration in nontaste tissue (Kleyman and Cragoe 1988; Labelle et al. 1984; Li et al. 1983; Orlowski 1993). In the case of 4-AP, 5 mM was chosen because this concentration has been reported to produce about one-half the maximal suppression of responses elicited to KCl and NH₄Cl (Kim and Mistretta 1993). The
present stimulation procedure was adopted to avoid drug concentrations and durations that may disrupt membrane components other than the ones targeted by the drug, and can be reversed relatively quickly without compromising spontaneous neural activity. A more detailed description of the rationale for adopting a procedure that involves the addition of a drug to the stimulus during sustained neural activity has been outlined previously (Lundy et al. 1997). Table 1 shows that the many brief presentations of the antagonists used in the present study were without
any measurable effect on neural sensitivity to the standard stimuli. The four standards were presented before and after testing a drug on responses to both concentrations of each salt.

Each salt concentration was presented twice and in an ascending order. In the experiments with amiloride and DMA, both applications consisted of a 15-s stimulation period followed by a 1.5- to 2.5-min water rinse. During the first presentation, the stimulus was applied continuously for 15 s, whereas the second presentation consisted of three consecutive segments with uninterrupted stimulus flow over the entire 15-s stimulation period. Specifically, a salt was presented alone during the first 5 s followed by the salt mixed with a drug for the next 5 s and, finally, the salt concentration alone again for the last 5 s (15 s total). See Fig. 7 for an example of the stimulation procedure.

In the experiments with 4-AP, an identical stimulation procedure was used with the exception that stimulus duration was 30 s, and each of the three segments in the second presentation were of 10-s durations. In the first few experiments with 4-AP, the shorter stimulating procedure described above for amiloride and DMA was used. It was noticed that the presence of 4-AP inhibited responses to KCl and NH4Cl in HCl-generalist neurons, but only during the last 5 s of the response, when the salt was presented alone again. Thinking that 4-AP might require >5 s to inhibit responses during the stimulation period in which it was mixed with a salt solution, the longer 10-s duration was employed.

The completion of an ascending salt concentration series was followed by a 5-min water rinse, after which a different salt series was presented. The order of salt series presentation was random (e.g.,
same temperature. At each new adapting temperature, a period of 3 min elapsed before application of the chemical stimuli. Each chemical stimulus was presented for 15 s and followed by a 120-s water rinse.

Data reduction and analysis

Response magnitudes were measured by counting the number of spikes during each stimulation segment (5 s for amiloride and DMA, 10 s for 4-AP) minus the baseline rate. To assess the effects of an antagonist, a paired samples t-test was conducted with the response rates during seconds 6–10 in both the first and second stimulus presentations as the paired samples. In the case of the temperature experiments, the difference between each neuron’s 15-s average response to a chemical stimulus and its 5-s average baseline rate immediately before stimulation was calculated at each temperature condition. Two-way ANOVAs were performed to detect significant differences between the varying temperature conditions. In some instances, post hoc contrast analyses (least significant difference method) were used to determine the source of the differences. Response rates to cooling from 35 to 10°C were measured by counting the number of spikes in 1-s bins during the temperature decrease. P values <0.05 were considered significant.

RESULTS

Figure 7, A and B, show examples of the stimulation procedures and the effect of 1 μM amiloride on the response of a NaCl-specialist neuron to 0.075 M NaCl. The response rate evoked by 15 s of stimulation with only the salt solution is shown in the top trace (A). The bottom trace (B) is the response rate to the same stimulus, but the 15-s stimulation period is broken up into three consecutive 5-s periods of stimulation. Amiloride was mixed with the 0.075 M NaCl solution during seconds 6–10 (2nd 5 s), and this portion of the response was nearly eliminated. It can be seen that the inhibitory effect of amiloride was reversed quickly when the stimulus switched back to NaCl alone during the last 5 s of the response (seconds 11–15). The ability of each drug to inhibit the response of geniculate neurons to both concentrations of NaCl, KCl, and NH₄Cl was assessed using the above stimulation procedures.

Amiloride and DMA

The responses of NaCl-specialist neurons to NaCl in the absence and presence of 1 μM amiloride (n = 5) and DMA (n = 6) are shown in Fig. 8. Response rates to 0.075 M NaCl were reduced by amiloride (t = 4.49, P = 0.01) and DMA (t = 3.55, P = 0.01), which was reversed quickly (last 5 s; P values >0.6). The mean percent control response was 44 ± 17% for amiloride and 45 ± 0.08% for DMA. The ability of increasing NaCl concentration (e.g., 0.3 M) to reduce the

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The values in parentheses are the significance values for the paired t-tests between stimulus presentations 1 and 2. P values <0.05 were considered significant.
The results of the study are as follows:

**4-AP**

A consistent effect on the responses of NaCl-specialist neurons ($n = 3$) to 0.075 M NaCl, like those obtained with amiloride and DMA, was not observed in the presence of 4-AP (P values >0.23). Surprisingly, 4-AP was without effect on the responses of HCl-generalist neurons to KCl and NH$_4$Cl ($n = 9$). Unfortunately, the present study was unable to test the effects of 4-AP in NaCl-generalist neurons. The salt responsiveness of the single QHCl-generalist neuron tested with 4-AP was unaffected. The only effect of 4-AP was that its activity 1.1 spikes/s over the baseline rate. A significant increase in the response rate to 5 mM 4-AP was evident in 5 of the 11 HCl-generalist neurons tested (mean discharge: 1.3 ± 0.4 spikes/s). This K$^+$ channel antagonist evoked the largest response in the single QHCl-generalist neuron tested, averaging 5.9 spikes/s over the 20-s stimulation period.

**Thermal sensitivity**

Shown in Fig. 9 are the mean responses of NaCl-specialist, NaCl-generalist, and HCl-generalist neurons to a 1°C/s temperature decrease from 35 to 10°C. The black bar below the 35 on the temperature axis indicates the onset of the temperature decrease. The 5-s baseline rates before the thermal change in each neuron group are also shown. The HCl-generalist neurons were by far the most sensitive to a decrease in temperature; their activity increased above baseline rate by 10.220.32.247 on May 31, 2017 http://jn.physiology.org/ Downloaded from 6 s after the temperature began to drop (29°C). Gradual cooling elicited a significant response in 20 of the 21 HCl-generalist neurons, whereas only 2 of the 10 NaCl-specialist neurons and 1 of the 5 NaCl-generalist neurons responded to cooling above the baseline rate. The sucrose-specialist ($n = 2$) and QHCl-generalist neurons ($n = 2$) tested were unresponsive to cooling.
Adaptation temperature and chemical stimulation

Shown in Fig. 10 are the response rates of NaCl-specialist, HCl-generalist, and QHCl-generalist neurons to 0.5 M sucrose, 0.1 M NaCl, 0.01 M HCl, and 0.02 M QHCl at 35, 25, and 15°C. Although HCl-generalist neurons responded to gradual cooling of the tongue, the baseline spontaneous activity when adapted to cooler temperatures was the same as or lower than the baseline spontaneous activity at 35°C. In fact, post hoc analysis (LSD method) of the main effect for temperature ($F_{2,18} = 5.5$, $P$ value $= 0.01$) indicated that the spontaneous activity following tongue adaptation to 35 and 25°C water was similar ($P$ value $= 0.60$), but greater than that for adaptation to 15°C water ($P$ values $\leq 0.01$). The baseline rate was similar following adaptation to the three different temperatures in NaCl-specialist ($F_{2,6} = 1.7$, $P$ value $= 0.25$) and QHCl-generalist neurons ($F_{2,3} = 5.4$, $P$ value $= 0.1$).

In this sample of NaCl-specialist neurons, it appears that temperature changes in the range of 35–15°C were without effect on the discharge rate to 0.1 M NaCl ($F_{2,36} = 0.04$, $P = 0.95$). The discharge rates to the standard stimuli in HCl-generalist neurons, on the other hand, were influenced by the temperature at which the receptors were adapted and the taste stimuli were delivered ($F_{2,84} = 22.2$, $P < 0.01$). A post hoc test revealed that the order of stimulus effectiveness was 35°C $>$ 25°C $>$ 15°C ($P$ values $\leq 0.04$). Although only two QHCl-generalist neurons were tested, an ANOVA revealed a significant main effect for temperature ($F_{2,24} = 8.9$, $P < 0.01$), the order of stimulus effectiveness was 35°C $=$ 25°C $>$ 15°C ($P$ values $< 0.02$). It is noteworthy that the response profile of the HCl- and QHCl-generalist neurons to the standard stimuli were unaffected by lowering temperature. The influence of temperature on the responses of sucrose-specialist and NaCl-generalist neurons to chemical stimuli awaits future investigation.

### TABLE 2. Discharge rate of neuron types to the antagonists

<table>
<thead>
<tr>
<th>Antagonist</th>
<th>1 μM Amiloride</th>
<th>1 μM DMA</th>
<th>5 mM 4-AP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose-specialist</td>
<td>0 ± 1 (5)</td>
<td>0 ± 0.2 (6)</td>
<td>0.2 ± 0.1 (4)</td>
</tr>
<tr>
<td>NaCl-specialist</td>
<td>0 ± 0.5 (5)</td>
<td>0.1 ± 0.3 (3)</td>
<td>0.7 ± 0.6 (11)</td>
</tr>
<tr>
<td>HCl-generalist</td>
<td>0.3 ± 0.3 (9)</td>
<td>0 ± 0.5 (4)</td>
<td>5.9 (1)</td>
</tr>
<tr>
<td>QHCl-generalist</td>
<td>0.1 (1)</td>
<td>0.1 (1)</td>
<td>11 (1)</td>
</tr>
</tbody>
</table>

Values are means ± SD with number of neurons in parentheses. The drugs tested are given in the 1st row and the neuron categories are given in the 1st column. DMA, 5-(N,N-dimethyl)-amiloride; 4-AP, 4-aminopyridine.
In over 60 yr of research on the peripheral gustatory system, this is only the second study to examine the neural response properties of geniculate ganglion neurons to controlled chemical stimulation of the tongue in rats. Current knowledge of peripheral coding mechanisms in taste comes largely from single fiber studies of cut axons limited by the length of time available for viable recording. Although relatively inaccessible and difficult to expose surgically, we took advantage of the ganglion cell preparation to obtain stable recordings over long periods to characterize the responses of geniculate neurons in four ways. Again because this was only the second study of its kind, we first examined ganglion cell responses to traditional stimuli (sucrose, NaCl, HCl, and QHCl) as means to appreciate the behavior of geniculate ganglion neurons in the context of the broader literature based on single fiber responses to the same stimuli. Second, we expanded the traditional stimulus array to include two concentrations each of KCl and NH₄Cl, as well as NaCl, to determine how the salt-sensitive chorda tympani nerve discriminates from among these salts. Third, we used three pharmacological antagonists of epithelial ion transport to explore salt transduction mechanisms for NaCl, KCl, and NH₄Cl. And finally, we varied stimulus and adaptation temperature to examine responses to cooling and the influence of temperature on chemical sensitivity. This four-pronged study provided unequivocal confirmation of chorda tympani fiber types with a broader and clearer definition of their unique response characteristics than was previously elucidated from single fiber studies.

**Standard stimuli and salt taste**

Like prior investigations (Boudreau et al. 1983; Frank et al. 1983, 1988; Hellekant et al. 1997a,b; Ninomiya et al. 1982), we demonstrated that ganglion cell neurons of the chorda tympani nerve were divisible into separate groups that responded uniquely to the standard stimuli. Our cluster analysis segregated the neurons into three main groups of best stimulus activation, sucrose, HCl, and NaCl. Closer examination of the NaCl response profiles indicated that three subgroups could be identified. We uncovered five groups while most of the prior studies identified only four using a similar array of stimuli. Two of our groups were narrowly tuned, and three were broadly tuned. Sucrose-specialists and NaCl-specialists responded best to sucrose and NaCl, respectively, and little if at all to the other stimuli. The three broadly tuned neuron groups responded well to salt, acid, and alkaloid stimuli but in different proportion. HCl-specialists responded significantly more to HCl than the other stimuli; QHCl-specialists responded significantly more to quinine hydrochloride. NaCl-specialists, like NaCl-specialists, responded best to NaCl, but also responded well to HCl and QHCl. The NaCl-specialist neuron class had not been considered a discrete group previously.

Because salt sensitivity is a dominant feature of the chorda tympani nerve, it is particularly instructive to know the way in which this one nerve distinguishes among three behaviorally distinct monochloride salts. Surprisingly, all five neuron groups expressed a unique sensitivity profile to NaCl, KCl, and NH₄Cl. Compared to the other four groups, sucrose-specialists were relatively unresponsive to all three salts except in a few instances when stimulated weakly by the highest 0.3 M concentration. NaCl-specialists were highly responsive to 0.075, 0.1, and 0.3 M concentrations of NaCl in a dose-response manner but were relatively unresponsive to equimolar concentrations of KCl and NH₄Cl. NaCl-specialists could, however, be activated weakly by 0.3 M KCl and NH₄Cl particularly after prolonged stimulation. HCl-specialists responded well to all three salts, but to NH₄Cl at twice the response rate than the other two stimuli. NaCl-specialists and QHCl-specialists were similar to each other, but different from the other groups, insofar as they responded equally well to all three salts across two stimulus concentrations. Thus among the five input channels of the chorda tympani nerve, it appears that NaCl-specialists and HCl-specialists are capable of responding differentially to NaCl, KCl, and NH₄Cl, and likely play a critical role in discrimination between these stimuli by rats.

As indicated previously, there is only one other study in which extracellular recordings were obtained from an intact...
The present results are largely in concert with the findings reported in that earlier study. Boudreau and his colleagues (1983) identified four groups of geniculate neurons: salt-responsive, acid-responsive, amino acid–sugar responsive, and alkaloid-responsive. Very little detail was given about the latter two groups other than to report their existence. It may be that the amino acid–sugar- and alkaloid-responsive neurons correspond to the present sucrose-specialist and QHCl-generalist neurons. Similar to Boudreau’s salt-responsive neurons using only 0.05 M salt concentrations, NaCl-specialists exhibited a high degree of specificity responding robustly to 0.1 M NaCl and little if at all.

**FIG. 10.** Shown are the response rates to 0.5 M sucrose, 0.1 M NaCl, 0.01 M HCl, and 0.02 M QHCl in NaCl-specialist, HCl-generalist, and QHCl-generalist neurons at different temperatures. Responses: □, 35°C; □, 25°C; □, 15°C.
to equimolar concentrations of KCl and NH₄Cl. In the present study, we also demonstrated that some NaCl-specialist neurons responded weakly to KCl and NH₄Cl, but only when the concentration was 0.3 M. In fact, the response rate to 0.3 M KCl and NH₄Cl in this neuron group increased two- to threefold with extended stimulation up to 30 s (data not shown). The instability of some salt-responsive neurons reported in the earlier study was not observed in the present experiments.

Similarities may also be found between the two studies with respect to acid-responsive neurons. Both report that the sensitivity of this neuron group to be broad but dominated by acid stimuli. Moreover, there is agreement on the greater sensitivity of acid-responsive neurons to NH₄Cl compared with NaCl and KCl. It was found in the present study that 0.075 M NH₄Cl was as stimulatory as 0.01 M HCl, whereas 0.3 M NH₄Cl was more effective than either of the other two. The greater sensitivity of acid-responsive neurons to NH₄Cl compared with NaCl and KCl may be due to NH₄⁺ → NH₃⁺ + H⁺ (acid).

Regardless of whether the recordings came from an intact ganglion or from cut axons in the afferent nerve (Frank et al. 1983, 1988; Hellekant et al. 1997a,b; Ninomiya et al. 1982), chorda tympani afferents can be meaningfully separated into at least four groups. For example, Frank et al. (1983) divided chorda tympani neurons into sucrose-best, NaCl-best, acid-best, and quinine-best groups according to the stimulus eliciting the largest response. Frank’s classification scheme corresponds to our sucrose-specialists, NaCl-specialists, HCl-generalists, and QHCl-generalists, respectively. In our sample of 73 geniculate ganglion neurons, 11% were sucrose-specialists, 25% NaCl-specialists, 45% HCl-generalists, and 3% QHCl-generalists. The 2:1 ratio of HCl-generalists to NaCl-specialists differs from the 1:1 ratio seen in earlier studies using only male rats (Frank et al. 1983, 1988) or male hamsters (Hettinger and Frank 1990). In the present study, twice as many HCl-generalist neurons came from males (n = 22) than from females (n = 11). It is therefore unlikely that sex was responsible for the proportional difference between studies. However, combining NaCl-generalists (n = 18) with NaCl-specialists (n = 12) would almost equal the numbers of HCl-generalists (n = 33) in the present study.

In the present study, we identified a fifth group of chorda tympani neurons, NaCl-generalists, with unique chemical sensitivity and response properties unlike those of the other four groups. A few studies have reported nonselective NaCl-best fibers in rodents, but no study has systematically characterized their unique features (Frank et al. 1983; Ogawa et al. 1968; Pfaffmann 1955). NaCl-generalists responded best to NaCl, but unlike NaCl-specialists, they were generally responsive to HCl and QHCl. Furthermore, NaCl-specialists responded robustly to NaCl and little if at all to KCl and NH₄Cl, whereas NaCl-generalists responded equally to these three salts. These characteristics are in agreement with a recent single axon study of the chorda tympani nerve in chimpanzees (Hellekant et al. 1997b). This study reported a subset of NaCl-best fibers (Na-K subcluster) that responded about equally to NaCl and KCl and were also responsive to HCl and QHCl.

Several response characteristics of the NaCl-generalist neurons also differed from those commonly associated with acid-best neurons. For HCl-generalists, 0.01 M HCl was the most effective stimulus among the four standards, and the temporal response pattern to this stimulus was unique. Unlike NaCl-generalists, the response of HCl-generalists to NH₄Cl was twofold greater than the responses to NaCl and KCl. Additionally, sucrose was a relatively ineffective stimulus for NaCl-generalists, whereas it evoked a weak but significant response from 45% of the HCl-generalists. A characteristic common to all generalist neurons was a higher rate of spontaneous activity while 35°C water flowed over the tongue (Ogawa et al. 1968).

**Antagonists of epithelial ion transport**

The present results with amiloride are in general agreement with the findings from the two prior studies of chorda tympani axons in rodents. In the earlier studies, amiloride was reported to be a potent inhibitor of the response rate to NaCl in hamster N-units (Hettinger and Frank 1990) and in rat high-amiloride-sensitive fibers (HAS) (Ninomiya and Funakoshi 1988). However, amiloride has also been shown to suppress responses to 0.01 M HCl in hamster chorda tympani (Hettinger and Frank 1990) and nucleus of solitary tract N-units (Boughter and Smith 1998), whereas, in rat HAS axons, amiloride inhibited responses to KCl concentrations >0.1 M (Ninomiya and Funakoshi 1988). Other studies also support the idea that amiloride suppression of neural taste responses may not be specific to just Na⁺ and Li⁺ stimuli (Gilbertson et al. 1993; Lundy and Contreras 1997; Lundy et al. 1997; Minear et al. 1996), as originally reported in whole nerve experiments (Brand et al. 1985; Heck et al. 1984; Hellekant et al. 1988). In the present study, amiloride only suppressed responses to NaCl in NaCl-specialist neurons. Direct comparisons between the single axon studies using amiloride must entertain species differences, but also the differences in the categorization of neuron groups. For example, several of Ninomiya’s and Funakoshi’s HAS fibers responded well to 0.01 M HCl. This suggests that some neurons in the HAS group may have been NaCl-generalists instead of NaCl-specialists according to our classification system.

Amloride antagonism of NaCl transduction mechanisms is most apparent in narrowly tuned neurons highly responsive to NaCl in rats and hamsters. Although the use of pharmacological blockers in conjunction with extracellular neural recordings is an indirect assessment of synaptology and receptor cell physiology, it seems reasonable to infer that taste receptor cells that express functional epithelial Na⁺ channels synapse exclusively with this physiological group of neurons. If the generalist neuron types are in synaptic contact with the same receptor cells, then amiloride should have inhibited the NaCl responses in these neurons. One is left to conclude that NaCl- and HCl-generalist neurons are not in synaptic contact with receptor cells that express functional epithelial Na⁺ channels. The receptor cells innervated by broadly tuned neurons may either utilize a different transduction mechanism for NaCl or amiloride was unable to gain access to the receptor sites.

Two previous studies have examined the inhibitory action of DMA on whole chorda tympani responses to salt stimulation. In rats, DMA suppressed nerve responses to NaCl, KCl, and NH₄Cl (Lundy et al. 1997), whereas in gerbils antagonists of the Na⁺/H⁺ exchange pathway were without effect on chorda tympani responses to salt stimulation (Schiffman et al. 1990). The present results are consistent with the prior study in rats, insofar as DMA suppressed responses to 0.075 M NaCl in NaCl-specialist neurons. Although identical procedures were used, these two studies are in conflict with regard to the
specificity of DMA suppression. Nevertheless, our studies of whole nerve and single-cell activity show DMA to be an antagonist of salt taste responses.

DMA is a relatively specific antagonist of $\text{Na}^+$/H$^+$ exchange (Kleyman and Cragoe 1988; LaBelle et al. 1984), showing very little if any inhibition of epithelial $\text{Na}^+$ channels at low mi-
cromolar concentrations in nontaste epithelia (Kleyman and Cragoe 1988). We assume that these pharmacological results apply to taste epithelia, as well. With this assumption in mind, there is evidence to support the notion that Na\(^+/H^+\) exchangers play a role in salt taste transduction. The present single-cell results suggest that NaCl-specialist neurons may be in synaptic

![Three-dimensional representations of stimulus relationships](image-url)
contact with receptor cells whose membranes contain Na\(^+\)/H\(^+\) exchange proteins. The presence of a Na\(^+\)/H\(^+\) exchange protein in taste cell membranes must be verified using other techniques such as patch-clamp recording or immunocytochemistry. If present on membranes of taste receptor cells, they may be coexpressed with epithelial Na\(^+\) channels.

The present experiments were unable to uncover a single neuron basis for the inhibition of KCl and NH\(_4\)Cl responses by 5 mM 4-AP observed in two prior experiments of integrated activity (Kim and Mistretta 1993; Simon et al. 1988). Kim and Mistretta (1993) showed that 5 mM 4-AP inhibited the integrated responses of the chorda tympani nerve to a range of KCl and NH\(_4\)Cl solutions in rats (0.025–0.25 M). In the present study, 4-AP was unable to inhibit the responses to any salt in NaCl-specialist and HCl-generalist neurons. This drug was, however, stimulatory in HCl- and QHCl-generalists (Table 2).

**Taste-temperature interaction**

The present study confirmed that there is a subset of chorda tympani neurons that are dually sensitive to cooling and chemical stimulation of the tongue (Ogawa et al. 1968; Yamashita et al. 1970). Decreasing the temperature of water flowing over the tongue by 1°C/s from the adapting temperature of 35°C down to 10°C evoked a robust response in HCl-generalist neurons. Ogawa and colleagues (1968) have reported previously a positive correlation between HCl, QHCl, and cooling. The thermal decrement used in the present study was without effect on NaCl-specialist, NaCl-generalist, QHCl-generalist, and sucrose-specialist neurons. Thus different subsets of geniculate neurons were sensitive to just chemical stimulation, to both chemical stimulation and cooling, but none were only sensitive to just cooling as reported in two earlier studies (Kosar and Schwartz 1990; Ogawa et al. 1968).

The present results agree with prior findings that single neuron responses to NaCl decreased as the adapting and stimulus temperature decreased below 30°C (Fishman 1957; Ogawa et al. 1968; Yamashita et al. 1970). As shown in the present study, the responses to NaCl as well as to sucrose, HCl, and QHCl were progressively reduced when assessed at 35, 25, and 15°C. Moreover, the temperature-mediated reduction in geniculate neuron responses to chemical stimulation was obvious in the HCl- and QHCl-generalists so examined. The taste responses of each NaCl-specialist neuron were the same at 35, 25, and 15°C. The influence of temperature on chemical sensitivity of sucrose-specialist and NaCl-generalist neurons awaits future investigation.

**Quality coding**

As a means to provide additional substantiating evidence for neuron types and to infer their role in quality coding, the present study used a multidimensional scaling procedure (MDS). Briefly, this statistical procedure produces a graphic representation of the similarities and differences in the pattern of stimulus-evoked neural activity. Stimuli that evoke a similar pattern of activity in a sample of neurons are represented by close proximity in the three-dimensional space. The interstimulus relationships across all ganglion neurons are shown first in Fig. 11A and are replotted in Fig. 12A. It appears that the distinct perceptual quality associated with sucrose can be accounted for entirely by the input from sucrose-specialist neurons (Fig. 11B). Without input from this group, sucrose remains distinguishable from the other stimuli only because it was relatively ineffective in the other neuron groups (Fig. 12B). Similarly, only the NaCl-specialists provide the information that clearly identifies NaCl as different from any other stimulus (Fig. 11C). The distinctiveness of NaCl is lost when the NaCl-specialists are excluded from the analysis (Fig. 12C). The ability of generalist neurons to provide input specifically about one class of stimuli is less clear (Figs. 11, D and E, and 12, D and E). However, the HCl-generalists alone appear to provide information that can distinguish NH\(_4\)Cl and the strongest NaCl concentration from the other stimuli, as well as from each other (Fig. 11D vs. Fig. 12D). The NaCl- and QHCl-generalists, on the other hand, seem to provide the input that distinguishes QHCl and the lowest KCl concentration from the other stimuli (Fig. 11E vs. Fig. 12E).

If neuron types function as labeled lines, then each type would be assumed to provide information only for a particular class of stimuli. The two criteria crucial for the labeled-line perspective of quality coding are clearly met by the sucrose- and NaCl-specialist neurons only. Specifically, these neurons form discrete physiological groups, and they appear to transmit information about only one class of taste stimuli. The present results and the results from two prior studies (Hettinger and Frank 1990; Ninomiya and Funakoshi 1988) show that the pharmacological action of amiloride is to block information transmitted solely along the input pathway of NaCl-specialists. When amiloride is used in behavioral studies, rats behave as if they can no longer discriminate NaCl from KCl and NH\(_4\)Cl (Hill et al. 1990; Spector et al. 1996). The future identification of antagonists that can interfere with the transduction processes of other physiological types will provide the means for a more direct demonstration of their functional role.

In conclusion, five neuron groups have been clearly identified based on extracellular recordings from the geniculate ganglion in rats. Four of these five neuron groups were similar to those identified previously using fine wire electrodes to record from single axons. Differences between the present five groups could be found in their sensitivity profile to sucrose, NaCl, HCl, QHCl, KCl, and NH\(_4\)Cl, their breadth of responsiveness, spontaneous firing rates, temporal response patterns, salt transduction mechanisms, temperature sensitivity, and their role in taste quality coding. Gustatory stimuli are parsed into discrete input pathways based on specific connections between lingual taste cells and afferent fibers, particularly specialist and generalist pathways. The specialist neurons may function as labeled lines that transmit information specifically about sweet-tasting and Na\(^+\)-containing compounds to the brain.

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