



## Research report

## Responses of single lingual nerve fibers to thermal and chemical stimulation

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**Abstract**

The goals of this study were to characterize the responses of: (1) thermally-sensitive fibers of the lingual branch of the trigeminal nerve to cooling from 35° to 10°C at a rate of 1°C/s; and (2) these neurons to a mid-range concentration of NaCl (150 mM), glucose (150 mM), citric acid (0.3 mM), and quinine-HCl (3 mM) at 35° and 25°C. A cluster analysis of 47 neurons' responses to cooling revealed two major groups and one minor group. Group 1 neurons ( $n = 19$ ) had a shorter latency, exhibited faster time-to-peak activity, and responded over a smaller range of temperature compared to Group 2 neurons ( $n = 22$ ). Group 3 neurons ( $n = 6$ ) exhibited the longest response latency and responded over a wider cooler range of temperature. Twenty-five out of thirty-one thermally-sensitive, non-tactile lingual neurons responded weakly to at least one chemical stimulus, with some neurons responding to 2, 3, or all 4 chemical stimuli. Group 1 neurons responded to more chemical stimuli at 35°C, while Group 2 neurons responded more at 25°C. Under their optimal temperature conditions, Group 1 and Group 2 neurons responded most often to citric acid and least often to glucose, with NaCl and Q-HCl eliciting an intermediate number of responses. As a whole, the responses of thermally-sensitive fibers to chemical stimulation were modest at best with an absence of chemical specificity. There was no evidence of a 'best' stimulus, although there was a suggestion of temporal coding.

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*Keywords:* Trigeminal; Electrophysiology; Common chemical sense; Taste**1. Introduction**

Two sensory nerves, the chorda tympani branch of the facial nerve (special sensory) and the lingual branch of the trigeminal nerve (general sensory), are responsible for relaying stimulus information from the anterior tongue to the brain. The chorda tympani nerve innervates taste buds located in the fungiform papillae [19]. The chorda tympani nerve is highly responsive to chemical stimuli: with most falling into four prototypical taste qualities perceived by humans as salty, sweet, bitter, and sour. The chorda tympani also responds to cooling and warming of the tongue [20]. While much research has been devoted to the conduction of neural signals by the chorda tympani nerve from taste cells to the central structures, relatively little research attention has been given to the potential role that the lingual nerve may play in taste coding.

Whole nerve electrophysiological recordings have shown the lingual nerve to be relatively insensitive to

prototypical tastants. Typically, high molar concentrations of chemicals have been required to elicit whole lingual nerve responses [6,7,22]. There are several factors that could contribute to the chemical insensitivity of whole nerve recordings. First, the lingual branch of the trigeminal nerve consists of four physiological classes of nerve fibers: mechanoreceptors, thermoreceptors, nociceptors, and proprioceptors [6,7,22] and only some of these types of fibers may be activated by chemical stimuli. Wang et al. [26] recorded the responses of individual rat lingual nerve fibers to a battery of chemical stimuli after classifying the fibers according to conduction velocity. C fibers and A $\delta$  fibers most likely responded to chemical stimulation, while A $\beta$  fibers did not respond to chemical stimulation. Biedenbach et al., [1] conducted an anatomical study of the fiber diameters in the cat lingual nerve showing fiber type distributions of ~5% unmyelinated C fibers ( $\leq 2 \mu\text{m}$  diameter), ~40% A $\delta$  fibers (2–5  $\mu\text{m}$  diameter), and ~55% A $\beta$  fibers ( $\geq 5 \mu\text{m}$  diameter). Wang et al. [26] confirmed this distribution in the rat lingual nerve. This means that less than half of the fibers in the lingual nerve are responsive to chemical stimuli. Furthermore, the unre-

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sponsive lingual fibers have the largest axon diameters. If the larger, chemically unresponsive A $\beta$  fibers are the primary fibers in contact with the recording electrode, it would not be unusual for whole nerve recordings of the lingual nerve to be insensitive to chemical stimulation.

A more recent study by Lundy and Contreras [17] demonstrated that relatively weak concentrations of quinine-hydrochloride (1.0 mM), citric acid (0.3 mM), and NaCl (100 mM) elicited significant responses in lingual fibers from rats. These responses were recorded from thirteen thermally-sensitive lingual fibers during 10 s presentations of the chemical stimuli at room temperature ( $\approx 25^\circ\text{C}$ ). Unlike differential responses of the chorda tympani nerve, it appeared that the lingual nerve responded similarly to qualitatively different chemical stimuli. It is intriguing that the lingual nerve might recognize chemical stimulation, yet not differentiate between stimuli. Lundy and Contreras [17] also reported that the lingual responses to the taste stimuli were highly correlated with warm water ( $45^\circ\text{C}$ ) responsiveness. Ogawa et al. [20] demonstrated that thermal stimulation of the chorda tympani nerve played a functional role in determining the quality of sensations from the tongue. There is the possibility that a similar relationship between thermal and chemical stimulation may exist for the lingual nerve.

In a subsequent study, Lundy and Contreras [18] found that responses of thermally-sensitive lingual fibers to menthol stimulation were temperature dependent. The fibers were more responsive to various menthol concentrations presented at  $35^\circ\text{C}$  than at  $25^\circ\text{C}$ . In addition, Lundy and Contreras [18] also reported that thermally-sensitive lingual neurons were separated into two groups based on their response characteristics to a  $1^\circ\text{C}/\text{s}$  decrease in water temperature from  $35^\circ\text{C}$  to  $10^\circ\text{C}$ . Response threshold, time-to-peak (threshold to maximum response), and range of sensitivity (75% of maximum response) were the three response characteristics used to separate the response patterns of the two groups of thermally-sensitive lingual neurons.

Previous electrophysiological studies of cutaneous receptors on primate skin have also described two groups of thermally-sensitive neurons based on rapid cooling of their receptive fields [2,4,5,12,15,16,21]. These studies discovered low-threshold cold receptors (LCRs) that responded to a rapid thermal transient with a short latency (low threshold) and a brief dynamic response ( $30^\circ\text{--}25^\circ\text{C}$ ) even though the temperature continued to decrease [2,4,5,12,15,21]. LaMotte and Thalhammer [16] identified another group of thermally-sensitive neurons as high-threshold cold receptors (HCRs). Unlike LCR neurons, HCR neurons only responded to thermal stimulation below  $27^\circ\text{C}$ . Lundy and Contreras [18] proposed that Group 1 and Group 2 neurons were the non-primate equivalents of LCRs and HCRs.

Because there has only been one prior study on this topic, the present study sought to re-evaluate the categorization of thermally-sensitive lingual fibers into two groups.

In addition, the present study examined the responses of these thermally-sensitive lingual neurons to chemical stimuli considered to elicit the sensations of salty, sweet, sour, and bitter. In as much as the lingual nerve response to menthol is temperature-dependent [18], the present study determined whether the responses of thermally-sensitive lingual fibers to chemical stimuli were also temperature-sensitive. Chemical sensitivity was assessed at  $35^\circ\text{C}$  (the approximate temperature of a rat tongue) or  $25^\circ\text{C}$  (room temperature).

## 2. Materials and methods

### 2.1. Subjects

Recordings were obtained from the lingual nerve of 6 adult male rats weighing 300–400 g (Sprague–Dawley, CrL:CD (SD) BR; Charles River Breeding Laboratories). Rats were housed in transparent plastic cages, a maximum of two animals 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. All preparations began approximately 4 h into the light phase.

### 2.2. Preparation

Rats were anaesthetized with urethane (1.5 mg/g body weight) administered in two intraperitoneal injections spaced 15 min apart. Supplementary doses of urethane (0.1 ml) were administered whenever the flexion withdrawal reflex could be evoked by pinching of the foot. Rectal temperature was monitored and maintained at  $36^\circ\text{--}38^\circ\text{C}$  throughout the experiment by a heating pad. The trachea was cannulated and a small suture was attached to the ventral surface to the tongue. During the preparation, the tongue was kept moist with cotton soaked in physiological saline. After placement in a nontraumatic head holder, the right lingual branch of the trigeminal nerve was exposed using a mandibular approach. The lingual nerve was transected proximally where it enters the foramen ovale and the perineurium was removed to the point where the lingual nerve joins the chorda tympani nerve. A small nerve strand was dissected from the nerve trunk and placed on a nichrome wire electrode. A similar indifferent electrode was placed in the underlying muscle tissue near the base of the nerve trunk for differential amplification ( $10,000\times$ ) of action potentials. The animal was grounded via the headholder. The action potentials were stored on a video recorder tape.

The tongue was slightly extended from the oral cavity and held in place by fixing the ventral tongue suture to the preparation table. Stimuli were presented to the anterior

portion of the tongue by computer-controlled stepping motors driving syringes to maintain a constant flow rate of 50  $\mu\text{l}$  per second. Throughout the experiment two stimulus bottles and a water rinse bottle were connected by polyethylene tubing and luer-lock adapters to separate input ports on the stimulus mixing platform. An independent valve within the mixing platform controlled the stimulus flow from each of the four input ports. These four input valves were controlled by a custom Power Macintosh 7100/80 computer program (designed by the technical support staff at Florida State University) that permitted rapid switching and/or mixing between the two stimulus and water channels while maintaining a continuous solution flow from the mixing chamber into the delivery faucet. From the delivery faucet, the solution flowed through a Peltier heat exchange device before being presented to the anterior portion of the tongue. The temperature of the solution can be held constant in a range of 5–50°C or changed within this range at a maximum rate of 1°C/s. A suction tube was placed underneath the tongue to remove the solution as it flowed off the tongue.

### 2.3. Stimulation protocols

#### 2.3.1. Neuron groups

Thermally-sensitive neurons were identified based on their response to a 1°C/s decrease in the solution temperature from 35°C to 10°C and then back again to 35°C. Once identified by their response to cooling, the responses of these thermally-sensitive neurons to chemical stimulation were examined at 35°C. After chemical stimulation, the

tongue was adapted to 25°C and then stimulated by a change in water temperature from 25°C to 10°C and then back again to 25°C in 1°C/s increments to assess the viability of the nerve activity. This was followed by the presentation of the chemical stimuli at the same 25°C adaptation. Fig. 1 depicts an example of a complete protocol series for a fiber. In between each stimulation there was at least a 90 s water rinse at the adapted temperature. Lingual fibers unresponsive to thermal stimulation from either temperature decrease (35° to 10°C or 25° to 10°C) were excluded from the study. Prior to chemical stimulation, each lingual fiber was tested for tactile sensitivity using an intermediate tactile stimulus. A No. 4.74 Von Frey hair was used to depress various positions distributed across the entire ipsilateral surface of the anterior tongue. Fibers containing a tactile sensitive component were noted.

#### 2.3.2. Chemical stimulation

Solutions were made from reagent grade chemicals dissolved in deionized–distilled water and were kept refrigerated when not in use. Chemical stimuli consisted of NaCl (150 mM), glucose (150 mM), quinine-HCl (3 mM), and citric acid (0.3 mM). The effect of temperature adaptation was assessed by presenting the four stimuli in two series. The first series was presented with the tongue adapted to 35°C and the second series was presented with the tongue adapted to 25°C. A period of 3–5 min elapsed after changing from 35°C condition to the 25°C condition to permit adaptation. Throughout the experiment, distilled deionized water at the appropriate temperature condition continuously flowed over the tongue to maintain the adap-

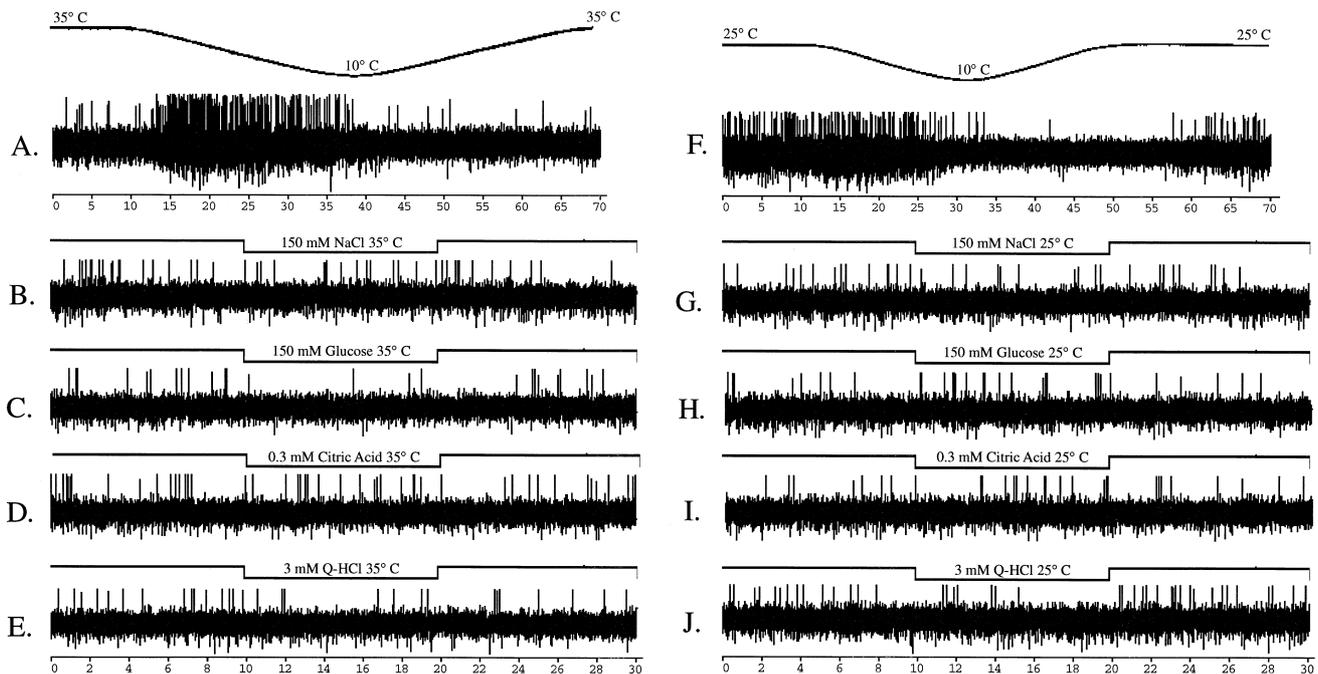


Fig. 1. An example of a neuron's response to the entire stimulation protocol. The solid bar above each neural response represents solution temperature (A, F) or the onset and offset of the stimulus (B–E, G–J).

tation temperature. Baseline neural activity was recorded during the water rinse for at least 20 s preceding each stimulus presentation. The stimuli were presented for 10 s and were followed by a water rinse of at least 90 s.

#### 2.4. Data reduction and analysis

The data were analyzed off-line on a Power Macintosh 7100/80 computer equipped with a GW Instrument 15  $\mu$ s data acquisition board and SuperScope II (SS II) data analysis software. The recorded responses of the thermally-sensitive lingual fibers were sampled by SuperScope II in 1  $\mu$ s periods and stored as a waveform. SuperScope isolated single unit responses from the multi-unit fiber recording based on amplitude and spike shape. The difference in single fiber activity during stimulation and the preceding 20 s baseline period was calculated. Average baseline activity approximated a normal distribution with individual neuronal activity ranging from 0.0 to 10.7 spikes/s with a mean rate of 3.9 spikes/s. The criterion for a neural response elicited by a stimulus was determined by a change in impulse frequency exceeding  $\pm 1.96$  standard deviations (SD) of the baseline activity.

### 3. Results

#### 3.1. Thermal sensitivity

The responses of 47 thermally-sensitive fibers to cool and warm water stimulation were studied. Three parameters seemed best to characterize the diversity of neural responses to cool and warm water stimulation; threshold, time-to-peak, and range of sensitivity [18]. Threshold was the time in seconds it took for the neuron to respond to cooling, exceeding 1.96 SD of the preceding 20 s baseline. The neuron's time-to-peak score was the time in seconds from the neuron's threshold to the neuron's maximum response. The neuron's range of sensitivity was the length of time that the neuron responded above 75% of its

maximum response. Each response parameter provided unique and defining information about the neuron's response. Threshold described response onset time, time-to-peak characterized the rise time of the response, and the range of sensitivity represented breadth of responsive across temperature.

Fig. 2 shows the responses of two single units to a change in temperature from 35°–10°–35°C in 1°C/s increments. The neuron in Fig. 2A had a threshold of 3 s, a maximum response of 5 s, and responded at or above 75% of its maximum response for 6 s. In contrast, the neuron shown in Fig. 2B had a longer latency of 12 s reaching its maximum response 9 s later. In addition, the neuron responded over a 7 s range that included the remaining cooling phase and some of the warming phase of the stimulation protocol.

Thermally-sensitive neurons were previously characterized into two groups based on the distribution of composite scores determined by the sum of a neurons' threshold, time-to-peak, and range of sensitivity in seconds [18]. However, there was no statistical basis to support linear summation of these measures as a composite score. In the present study, a K-means cluster analysis was used to identify neuron groups based on their individual response parameters rather than composite scores. Fig. 3 shows the results of the cluster analysis. The K-means cluster analysis showed that threshold ( $F_{21,44} = 3.2$ ,  $p < 0.05$ ), time-to-peak ( $F_{21,44} = 27.8$ ,  $p < 0.01$ ), and range of sensitivity ( $F_{21,44} = 31.4$ ,  $p < 0.01$ ) all made significant contributions in identifying three principal neuron clusters. There were two major clusters that accounted for 87% of the neurons (Group 1,  $n = 19$ ; Group 2,  $n = 22$ ) and a third minor cluster (Group 3,  $n = 6$ ). Fig. 3 shows that Group 1 and 2 neurons are relatively similar to each other yet separate from Group 3 neurons in the cluster analysis. Thus, additional analyses focused on the dominant two groups, while Group 3 neurons were addressed separately.

Relative to the rate of cooling, the average threshold was 4 s for Group 1 neurons and 7 s for Group 2 neurons.

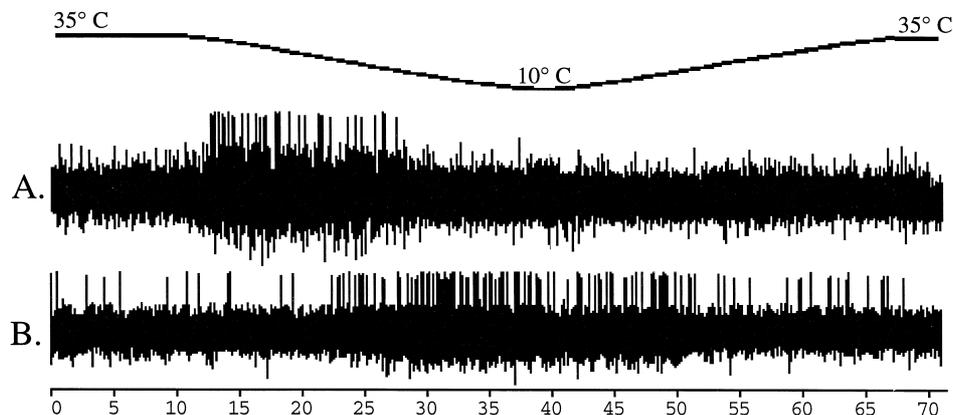


Fig. 2. The response of a Group 1 thermally-sensitive fiber (A) and a Group 2 thermally-sensitive fiber (B) to the 35°–10°–35°C stimulation sequence. The solid bar above the spike records depicts the solution temperature during adaptation and the 1°C/s temperature change.



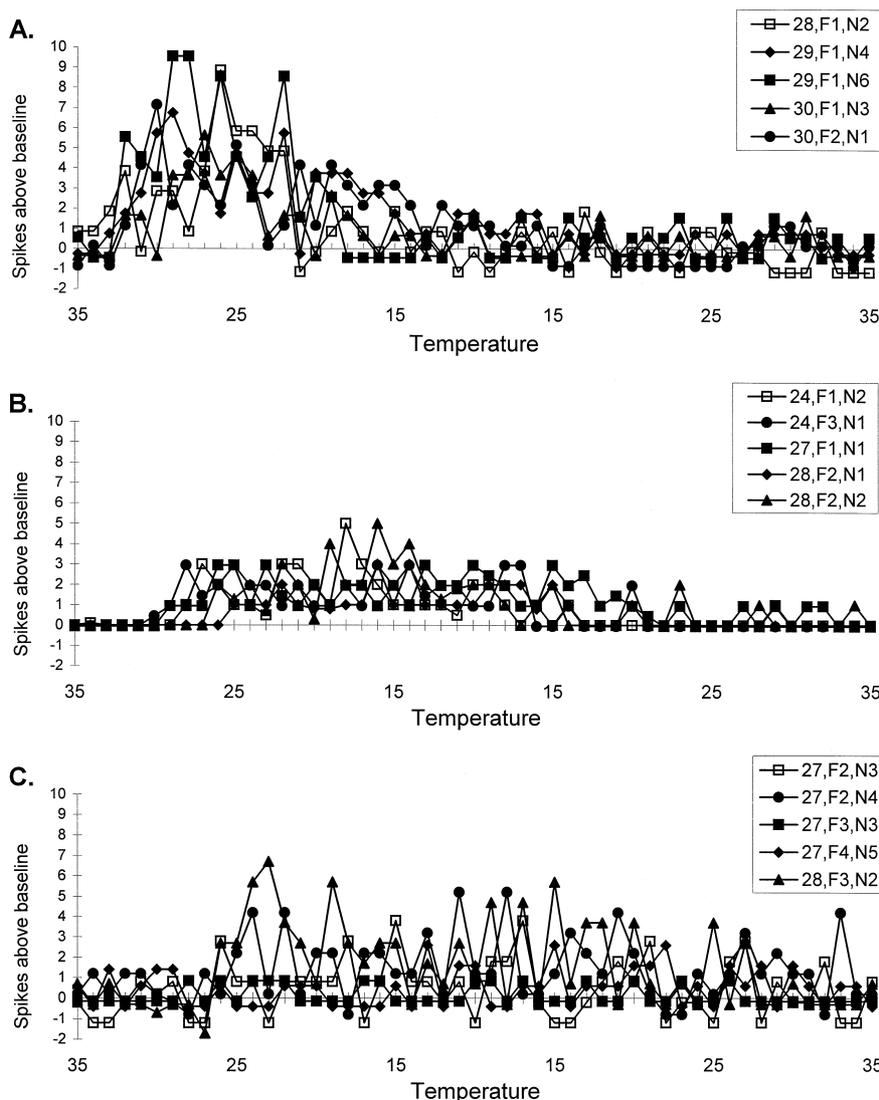


Fig. 4. Spikes/s relative to the 20 s baseline for 5 representative neuron's responses to the temperature stimulation from 35° to 10°C to 35°C. Each tick on the horizontal axis represents a 1°C/s change in solution temperature. The neurons are categorized as Group 1 response patterns (A), Group 2 response patterns (B), or Group 3 response patterns (C).

above baseline activity. This variable activity continued into the re-warming phase.

The average response profiles of all Group 1 (A), Group 2 (B), and Group 3 neurons (C) are shown in Fig. 5. The small standard error bars demonstrate the low variability of Group 1 and Group 2 neuron responses. The large variability of the Group 3 neuron responses was partly a factor of the low number of neurons in the sample confounded by the sporadic bursting response described above. Fig. 5 provides an accurate representation of the group differences in response latency, rise time, and range of responsiveness for each response profile. In addition, there was a difference in the overall magnitude of the responses, with Group 1 neurons having approximately twice the response magnitude of Group 2 and 3 neurons.

Group 3 neurons had substantially higher threshold (average = 10 s), time-to-peak (average = 14 s) and range

of sensitivity (average = 17 s) values than Group 1 and Group 2 neurons. Neurons ( $n = 2$ ) with similar Group 3 response characteristics were found previously [18] but were included in the Group 2 pool. We excluded Group 3 neurons from the analysis of chemical responsiveness because of the small sample size. It is, nevertheless, possible that Group 3 neurons may represent a third population of thermally-sensitive fibers that respond to a lower temperature range than either Group 1 or Group 2. In future studies, the pool of Group 3 neurons may be enlarged by decreasing stimulus temperature lower than 10°C.

### 3.2. Tactile sensitivity

Of the 41 neurons that were either Group 1 or Group 2, 10 neurons exceeded the 1.96 SD response criterion during tactile stimulation as well as to thermal stimulation. Of

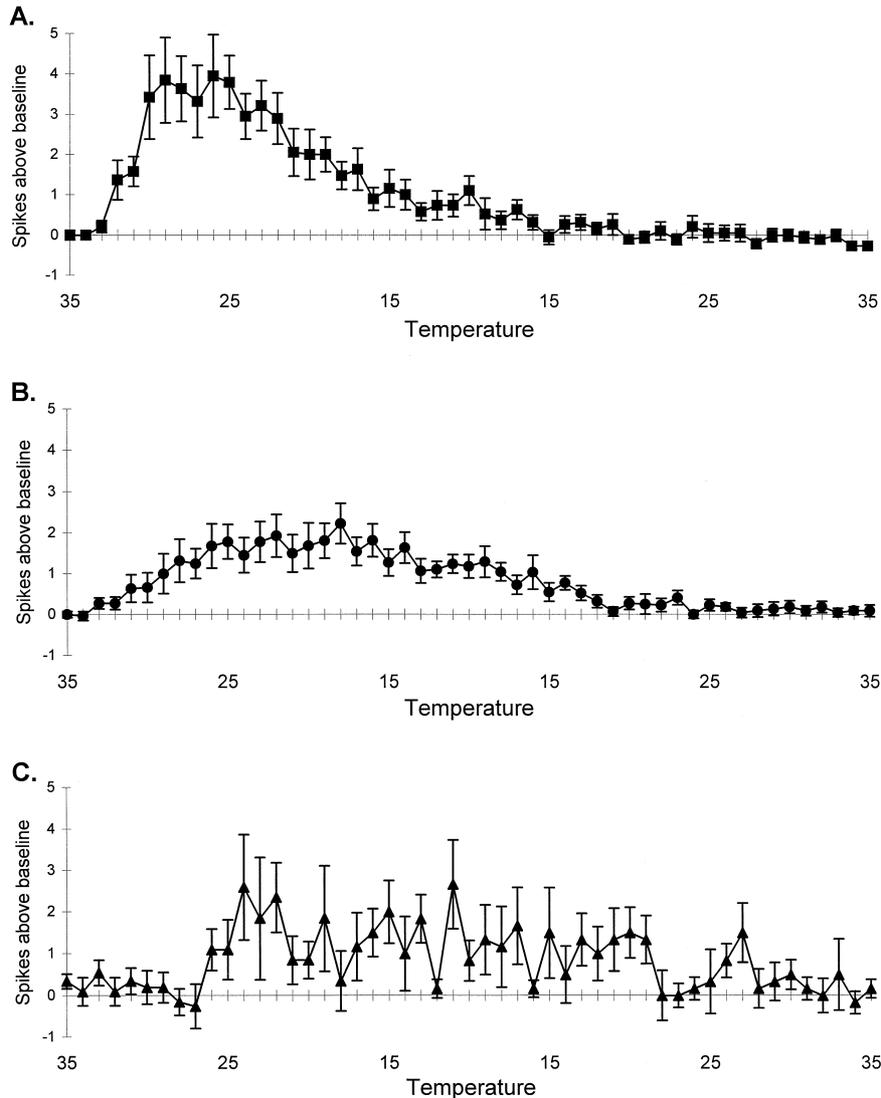


Fig. 5. Average spikes/s relative to the 20 s baseline activity for Group 1 neurons (A), Group 2 neurons (B), and Group 3 neurons (C) during the temperature stimulation from 35° to 10°C to 35°C. Each tick on the horizontal axis represents a 1°C/s change in solution temperature. Error bars represent the standard error.

these 10 neurons, 8 were from Group 1 and 2 were from Group 2. Six of the eight tactile-sensitive Group 1 neurons and both of the Group 2 neurons responded to chemical stimulation.

Using conduction velocity, Wang et al. [26] categorized lingual neurons into C, A $\delta$  cold fibers, or A $\beta$  fibers, and then examined their thermal, chemical, and tactile responsiveness. They showed that C fibers responded to thermal, tactile, and chemical stimulation, while A $\beta$  fibers were responsive only to thermal and tactile stimulation, and A $\delta$  cold fibers were responsive only to thermal and chemical stimulation. Since we did not measure conduction velocity, we do not know the distribution of C, A $\delta$ , or A $\beta$  fibers that were in the present sample. However based on their responsiveness to different stimulus modalities, it seems logical to infer that the 3 neurons which responded to thermal and tactile but not chemical stimulation were

probably A $\beta$  fibers. Similarly, the 8 neurons which responded to thermal, tactile, and chemical stimulation were most likely C fibers. Finally, the remaining neurons were most likely A $\delta$  fibers, responsive to thermal and chemical stimulation, but not to tactile stimulation. Consequently, the 10 tactile-sensitive neurons were considered a separate class and excluded in the general analysis of chemical sensitivity.

### 3.3. Responsiveness to chemical stimuli

#### 3.3.1. All non-tactile sensitive neurons

The criterion for a response to chemical stimulation was activity initiated within the first 3 s of stimulation that was  $\geq 1.96$  SD of the prior 20 s baseline. Using this criterion, 81% or 25 of 31 neurons responded to at least one tastant under either the 35° or 25°C temperature conditions. Of

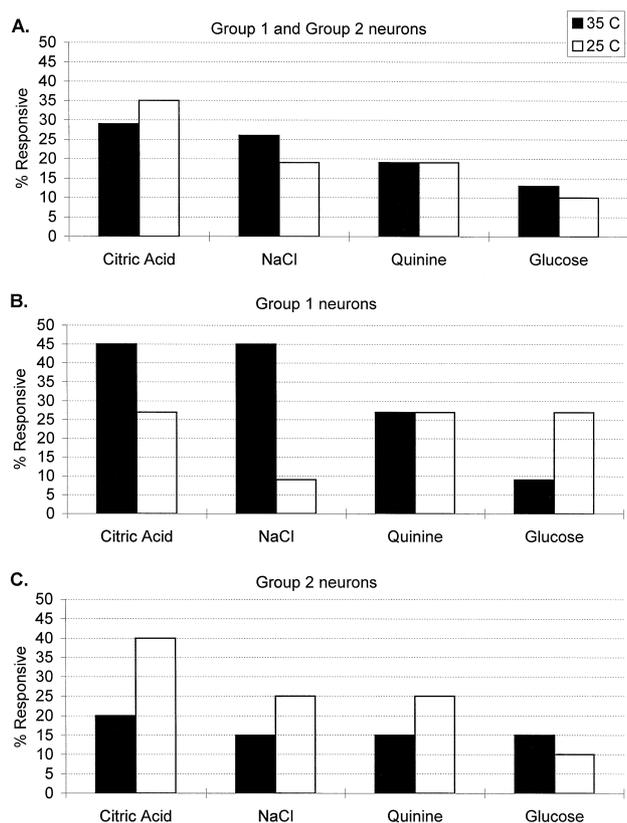


Fig. 6. The percentage of both Group 1 and Group 2 thermally-sensitive lingual neurons (A) that responded to each of the four chemical stimuli during either 35°C and 25°C temperature adaptation. Percent responsive neurons are shown as Group 1 (B) and Group 2 (C) in order to compare differences during stimulation at 35° and 25°C.

these 25 chemically sensitive neurons, 8 responded to only one stimulus and 17 neurons responded to more than one stimulus (4 responded to all 4 stimuli, 6 responded to 3 stimuli, and 7 responded to 2 stimuli).

Fig. 6A shows the percentage of neurons that responded to each chemical stimulus at 35° and 25°C. Nine of 31 neurons (29%) were responsive to citric acid at 35°C and 11 of 31 neurons (35%) were responsive to citric acid at 25°C. In contrast, fewer neurons were responsive to glucose at either 35°C (4 of 31 neurons) or 25°C (3 of 31 neurons).

Table 1 lists the proportion of neurons that responded to one or more chemical stimuli at 25° and 35°C. Across all neurons, there was no difference in the proportion of neurons responsive at 25°C (26 of 36 neurons) and at 35°C

(20 of 36 neurons). However, when chemical responsiveness was separated by neuron group, statistical differences emerged. The same proportion of Group 1 neurons (8/11) responded to chemical stimulation at 35° and 25°C. However, proportionally more Group 2 neurons responded to chemical stimulation at 25°C than at 35°C ( $F_{19} = 4.6$ ,  $p < 0.05$ ). There was also a significant difference in the overall responsiveness of Group 1 and Group 2 neurons to chemical stimulation across the temperature conditions ( $F_{30} = 7.36$ ,  $p < 0.01$ ). Group 1 neurons responded to 28% of all chemical stimulus presentations, while Group 2 neurons responded to 21% of the presentations. This may indicate that Group 1 neurons are slightly more sensitive to chemical stimulation than Group 2 neurons.

### 3.3.2. Group 1 neurons

Although proportionally the same number of Group 1 neurons responded to chemical stimulation at 35° and 25°C, Group 1 neurons responded to more stimuli at 35°C than at 25°C. The 4 chemical stimuli were presented once to each of the 11 Group 1 neurons resulting in 44 opportunities to respond at 35° or 25°C. Group 1 neurons responded to 32% (14/44) of the stimulus presentations at 35°C, but only 23% (10/44) at 25°C, suggesting that the chemical sensitivity of Group 1 neurons may be optimal at 35°C. This is consistent with their responses to cooling. Group 1 neurons had a lower temperature threshold and responded over a narrow temperature range.

The percent responsiveness of Group 1 neurons to chemical stimulation at each temperature is illustrated in Fig. 6B. Citric acid and NaCl elicited the most responses of Group 1 neurons at 35°C. Quinine evoked a moderate amount of responses, while glucose was relatively ineffective. The responsiveness of Group 1 neurons to citric acid and NaCl was much lower at 25°C, while quinine responsiveness was the same at both temperatures. Group 1 neurons were more responsive to glucose at 25°C.

### 3.3.3. Group 2 neurons

As previously mentioned, a significantly larger proportion of Group 2 neurons (14 of 20 compared to 9 of 20) responded to one or more chemical stimuli at 25°C than at 35°. In addition, two neurons responded to chemical stimulation only at 35°C, but eight neurons responded only to chemical stimulation at 25°C. The remaining 10 chemically responsive Group 2 neurons were activated by stim-

Table 1  
The effect of temperature adaptation on the responsiveness to chemical stimulation

	Temperature adaptation	
	35°C	25°C
All neurons	17 of 31 neurons responded 55%	22 of 31 neurons responded 71%
Group 1 neurons	8 of 11 neurons responded 73%	8 of 11 neurons responded 73%
Group 2 neurons	9 of 20 neurons responded 45%	14 of 20 neurons responded 70%

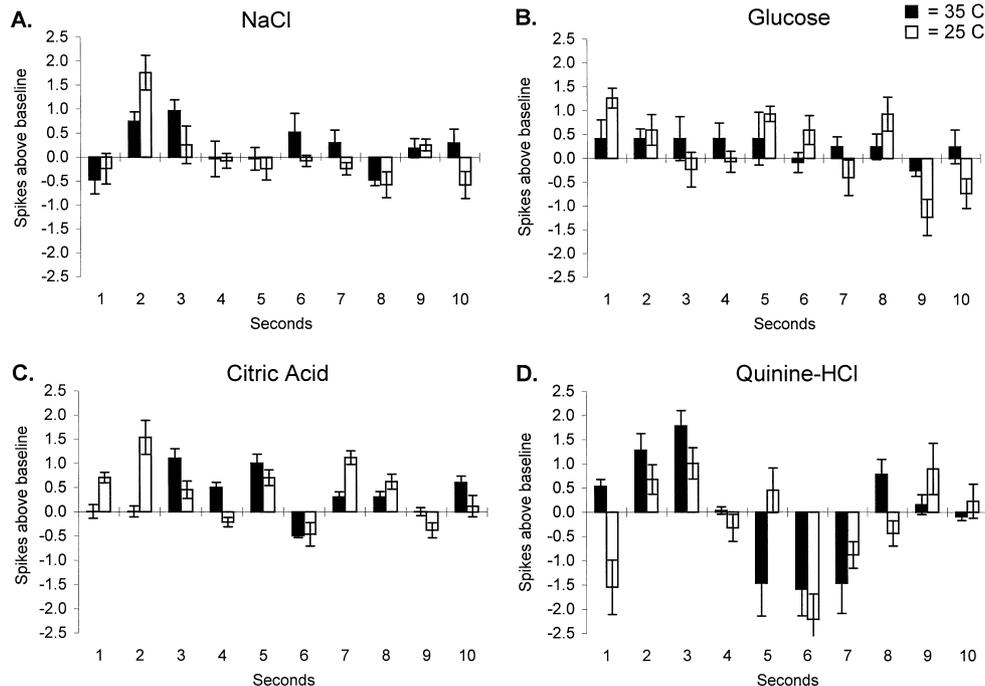


Fig. 7. The mean spikes/s of responsive Group 1 and Group 2 neurons during the 10 s presentation of chemical stimuli. The filled bars represent responses at 35°C and the unfilled bars represent responses at 25°C. Error bars represent the standard error.

uli at both temperatures. This suggests that the chemical sensitivity of Group 2 neurons may be better at 25°C rather than at 35°C. This is consistent with their responses to cooling. The range of sensitivity of Group 2 neurons spanned 28° to 14°C encompassing the 25°C adaptation temperature.

Fig. 6C shows the percent responsiveness of Group 2 neurons to chemical stimulation at 35° and 25°C. At 35°C, Group 2 neurons responded to 15–20% of the presentations of each stimulus. At 25°C, Group 2 neurons responded more often to citric acid than the other 3 stimuli.

### 3.4. Response magnitudes and temporal patterns

As noted above, chemical sensitivity varied with adapting temperature and neuron group. However, less than half of the neurons tested were responsive to any one chemical stimulus. Furthermore, there was no evidence of neurons responsive to particular or ‘best’ stimuli. Thermally-sensitive lingual neurons were not ‘tuned’ at least in terms of response frequency to particular chemical tastants as are the neurons of the special sensory taste nerves (chorda tympani, glossopharyngeal, and greater superficial petrosal). While lingual neuron responses to chemical stimulation were infrequent and of low magnitude, there appeared to be temporal differences in the responses evoked by the chemical stimuli during the 10 s presentation period.

Fig. 7 shows the mean response magnitudes in spikes/s minus the mean baseline activity of all chemically-responsive neurons to 10 s presentations of the stimuli at 35° and

25°C. The response magnitudes were small, less than 2 spikes/s change from baseline. As can be seen, the temporal response patterns of thermally-sensitive neurons were somewhat unique for each stimulus. In Fig. 7A, the excitatory response to NaCl at 35°C ( $n = 9$ ) and 25°C ( $n = 6$ ) was brief, peaking 2–3 s following stimulus onset with little or no activity during the remainder of the 10 s stimulation period. The response to glucose at 35°C ( $n = 6$ ) appeared to be low and sustained for the first 5 s of stimulation followed by a decrease in the response. The response to glucose at 25°C ( $n = 6$ ) was cyclic with periodic excitatory and no response periods. Similarly, Fig. 7C shows an intermittently excitatory response to citric acid at both 25°C ( $n = 12$ ) and 35°C ( $n = 10$ ). Quinine evoked an initial phasic excitatory response peaking in magnitude 3 s after stimulus onset which was followed a 3 s tonic inhibitory response at both 35°C ( $n = 8$ ) and 25°C ( $n = 9$ ).

## 4. Discussion

The present study investigated three related issues. First, we investigated the response patterns of thermally-sensitive lingual neurons to cooling and re-warming. Consistent with previous findings, the present results show that the response parameters; threshold, time-to-peak, and range of sensitivity segregated lingual neurons into two major clusters. Furthermore, we found a third group of thermally-sensitive neurons that was optimally responsive to cooling from 20° to 10°C. Second, we examined the responses of

thermally-sensitive lingual neurons to low-mid range concentrations of 4 chemical stimuli. The majority of the thermally-sensitive neurons responded to at least one chemical stimulus typically with differential temporal patterns. Lastly, we investigated whether tongue adaptation temperature affected the responsiveness of lingual neurons to chemical stimulation. We found that the tongue adaptation temperature played a role in the responsiveness of Group 1 and Group 2 neurons to chemical stimulation, but did not have a marked affect on response magnitudes.

#### 4.1. Thermal sensitivity

Cluster analysis of three response parameters to cooling from 35° to 10°C revealed two predominant neuron groups as well as a third less prevalent neuron group warranting further investigation. Although threshold, time-to-peak, and range of sensitivity, contributed significantly to the cluster analysis, only time-to-peak and range of sensitivity differed significantly between Group 1 and Group 2 neurons. Group 1 neurons exhibited rapidly adapting features, characterized by a sharp onset response that was short-lasting ceasing after 4 s even as stimulus temperature continued to decline. Group 2 neurons, on the other hand, responded with slowly adapting features. They gradually increased their activity reaching a plateau that spanned a range of 12° (26°–14°C). This may reflect their delayed response onset or Group 2 neurons may be more sensitive over a lower temperature range than Group 1 neurons.

The response patterns of Group 1 and Group 2 neurons are similar to the response characteristics of high-threshold cold receptors (HCRs) and low-threshold cold receptors (LCRs), respectively [5,16,21]. LaMotte and Thalhammer [16] suggested that the presence of two overlapping thermally-sensitive mechanisms provides a smooth transition between populations of neurons that vary in their ranges of temperature sensitivity. Although not examined in the present study, it is possible that the different responses of Group 1 and Group 2 neurons to cooling may be related to the neuron receptor's depth from the surface of the tongue rather than unique physiological properties of the neuron groups. For example, if Group 1 receptors were located relatively close to the tongue's surface, then they would tend to respond quickly to changes in solution temperature providing a short onset time. Relative to adaptation temperature, most thermally-sensitive neurons are stimulated by cooling and inhibited by warming [16–18,21]. So it is curious that Group 2 neurons (Fig. 4B Fig. 5B) continued to respond during the first 5 s of the re-warming phase (10°–15°C). Having their receptive membranes buried within the depths of the tongue and removed from its surface would account for Group 2 neurons' sluggish and persistent response into the first 5 s of the re-warming phase. While Group 1 receptors located closer to the surface of the tongue could adapt faster, showing a smaller range of sensitivity than deeper Group 2 receptors. It

should be noted, however, that Group 1 and Group 2 neurons differ not in threshold but in their rate of activation.

#### 4.2. Chemical sensitivity

About 83% of the Group 1 and Group 2 lingual neurons responded to at least one of the four chemical stimuli representing sweet, salt, sour, and bitter for the gustatory system. Only a single concentration of each stimulus was used but they were low to mid range concentrations commonly used to stimulate responses in the main special sensory taste nerves, the chorda tympani, greater superficial petrosal, and glossopharyngeal nerves. Typically, stimulus concentrations ten-fold higher above 1 M are required to elicit responses from the whole lingual nerve [22]. Similar to electrophysiological recordings from the main sensory taste nerves, the lingual nerve responses appeared to display differential temporal response patterns for each chemical stimulus (Fig. 7). However, unlike the main sensory taste nerves, there was no evidence that lingual nerve fibers had a strong preference for specific chemical stimuli. More neurons responded to citric acid than any other stimulus. The responses were modest (2–3 spikes/s about baseline) when compared to chorda tympani neurons (15–20 spikes/s above baseline) responses to taste stimuli [8,9]. Furthermore, in cases of multiple chemical sensitivity, the lingual neurons had the same modest firing rate to all stimuli. In contrast, the chorda tympani nerve consists of N-best, H-best, S-best, and Q-best fibers that respond better to specific stimuli compared to others. For example, S-best fibers respond best to sucrose, send best to NaCl, and little if at all to HCl or quinine [8,9].

There appeared to be an effect of tongue temperature adaptation on the chemical responsiveness of Group 1 and Group 2 neurons. Group 1 neurons were most responsive at 35°C and Group 2 neurons were most responsive at 25°C. Consequently, their chemical sensitivity seemed to match their sensitivity to cooling where Group 1 neurons were more responsive at temperatures near 35°C while Group 2 neurons were more responsive at temperatures near 25°C. These respective temperature conditions were considered more optimal temperature adaptations for either Group 1 or Group 2 neurons. There also appeared to be a consistent pattern of responsiveness to the chemical stimuli between the Group 1 and Group 2 neurons at their respective optimal temperature adaptation. The order and degree of responsiveness to chemical stimuli for Group 1 neurons at 35°C was similar to the Group 2 neurons' order and degree of responsiveness to stimuli at 25°C (Fig. 6B and C). Both Group 1 neurons at 35°C and Group 2 neurons at 25°C were most responsive to citric acid and least responsive to glucose. The tongue adaptation temperature did not have an affect on the magnitude of the chemical responses. Lundy and Contreras [18] found that menthol response magnitudes were threefold greater at 35°C than at 25°C. In

the present study, temperature did not influence the response magnitudes of lingual fibers to glucose, NaCl, citric acid, and quinine. This may suggest that menthol and the traditional taste stimuli activate lingual fibers through different receptive mechanisms.

Although lingual neurons responded similarly and modestly to all chemical stimuli, there appeared to be unique temporal patterns for each chemical stimulus. It would be imprudent to suggest, however, that lingual neurons are capable of transmitting taste quality information at the present time. Nevertheless, if one looks closely the lingual nerve responses are similar in kind, although not in magnitude, to chorda tympani responses. For example, the lingual fibers response to NaCl can be characterized as initially phasic followed by a steady state level near baseline. This is similar to the chorda tympani response to weak NaCl. The lingual fiber response to glucose is cyclical similar to the cyclic response of the chorda tympani to sucrose. The lingual fiber response to citric acid is relatively sustained like the chorda tympani response of H-best fibers [9]. The lingual fiber response to quinine is unique. The initial excitatory response followed by inhibition is different than the responses to the other stimuli and not seen in the chorda tympani. However, the lingual fiber response to quinine seems to be the most pronounced of all the chemical response patterns.

Although based on preliminary evidence, the suggestion that lingual fibers may transmit a temporal code for taste quality makes sense for two reasons. First, many studies of gustatory nerves have been conducted showing that discrimination and response sensitivity are impaired by taste nerve transections, but it has been extremely difficult to eliminate taste-mediated behavior [23,24]. While most studies only attempt one or two taste nerve transections in a given animal, one study to date has undertaken transection of all three special sensory taste nerves in examining taste function. Spector et al. [24], demonstrated that the combined transection of the chorda tympani, greater superficial petrosal, and glossopharyngeal nerve considerably reduced the sucrose concentration lick-rate response function, but did not eliminate an increase in lick rate to sucrose relative to water. This opens the possibility that taste-mediated behavior could be mediated by trigeminal signaling.

Anatomical studies provide additional support that lingual fibers may be involved in taste coding. In the anterior oral cavity, the lingual branch of the trigeminal nerve accounts for 75% of the innervation of the fungiform papillae and the chorda tympani nerve accounts for the remaining 25% [22]. The lingual nerve terminates in the lingual epithelium, subepithelial connective tissue, and papillary layer as well as being observed terminating in taste buds without forming synapses [13,14,28]. Several studies have reported the persistence of some taste pores in the anterior tongue following chorda tympani transection [10,25,29]. Hård af Segerstad et al. [11] along with Kinn-

man and Aldskogius [14] provided evidence that after chronic chorda tympani denervation, the lingual nerve may sprout branches that enter a fungiform papilla and maintain the taste bud. There have been no studies to date examining the chemical responsiveness of the lingual nerve following transection of one or more of the special sensory taste nerves. Furthermore, the rostral nucleus of the solitary tract receives overlapping afferent projections from gustatory VII and IX as well as from lingual V cranial nerves [3,27]. This first sensory relay nucleus may be a possible integration site for afferent neural signals from the trigeminal and special sensory taste nerves.

In conclusion, the present study demonstrated the existence of two groups of neurons based on their response characteristics to cooling from 35° to 10°C. The majority of these thermally-sensitive lingual neurons were responsive to chemical stimuli considered to elicit the sensations of salty, sweet, sour, and bitter. While tongue temperature adaptation affected the chemical responsiveness of Group 1 and Group 2 neurons, it did not have an effect on the magnitude of the chemical responses. Along with the electrophysiological data presented in this paper, the findings from nerve transection and anatomical studies are consistent with the notion of taste detection or quality coding in lingual nerve fibers. It is important to resolve in future studies the role of lingual afferents in taste-mediated behavior.

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