Chem. Senses 28: 45–55, 2003

# Stimulation of Bitterness by Capsaicin and Menthol: Differences Between Lingual Areas Innervated by the Glossopharyngeal and Chorda Tympani Nerves

**Barry G. Green[1,2] and Matthew T. Schullery[1]**

[1]The John B. Pierce Laboratory and [2]Department of Surgery (Otolaryngology), Yale School of Medicine, New Haven, CT 06519, USA

*Correspondence to be sent to: Barry G. Green, The John B. Pierce Laboratory, 290 Congress Avenue, New Haven, CT 06519, USA. e-mail: green@jbpierce.org*

## Abstract

Capsaicin is viewed as a purely chemesthetic stimulus that selectively stimulates the somatosensory system. Here we show that when applied to small areas of the tongue, capsaicin can produce a bitter taste as well as sensory irritation. In experiment 1, individuals were screened for the ability to perceive bitterness from capsaicin on the circumvallate papillae. Fifteen of 25 subjects who reported at least weak bitterness rated the intensity of taste, irritation and coolness produced by 100–320 μM capsaicin and 100–320 mM menthol applied via cotton swabs to the tip (fungiform region), the posterior edge (foliate region), and the dorsal posterior surface (circumvallate region) of the tongue. Sucrose, citric acid, sodium chloride and quinine hydrochloride were applied to the same areas to assess tastes responsiveness. On average, capsaicin and menthol produced 'moderate' bitterness (and no other significant taste qualities) in the circumvallate region, and weaker bitterness on the side and tip of the tongue. Sensory irritation from capsaicin was rated significantly higher at the tongue tip, whereas menthol coolness was rated higher in the circumvallate region. In experiment 2 we applied sucrose and quinine hydrochloride together with capsaicin to investigate the effects other taste stimuli might have on capsaicin's reported bitterness. As expected, adding quinine produced stronger bitterness in the circumvallate and fungiform regions, and adding sucrose significantly reduced the bitterness of capsaicin in the circumvallate region. Overall, the results suggest that capsaicin and menthol are capable of stimulating a subset of taste neurons that respond to bitter substances, perhaps via receptor-gated ion channels like those recently found in capsaicin- and menthol-sensitive trigeminal ganglion neurons, and that the glossopharyngeal nerve may contain more such neurons than the chorda tympani nerve. That some people fail to perceive bitterness from capsaicin further implies that the incidence of capsaicin-sensitive taste neurons varies across people as well as between gustatory nerves.

## Introduction

Capsaicin is the most widely used experimental probe of oral chemesthesis and the trigeminal system. Use of capsaicin as the prototypical chemesthetic stimulus stems not only from its ability to desensitize a specific class of trigeminal neurons (Jancso, 1960; Szolcsanyi, 1977; Buck and Burks, 1986; Green, 1989; Karrer and Bartoshuk, 1991; Liu *et al.*, 1996; Dessirier *et al.*, 2001a), but also from the assumption that it stimulates somatosensory neurons (principally pain fibers) without affecting gustatory neurons (Szolcsanyi, 1977; Hettinger and Frank, 1992). The discovery of a specific vanilloid receptor (Szallasi *et al.*, 1993; Caterina *et al.*, 1997; Tominaga *et al.*, 1998) strengthened the belief that capsaicin's excitatory effects in the mouth are limited to a specific type of trigeminal neuron that transmits information about heat and acidity.

However, some of the earliest psychophysical studies of oral chemesthesis gave evidence that capsaicin could evoke weak sensations of taste (Lawless, 1984; Lawless and Stevens, 1988). Probably because the taste ratings appeared to be variable and indistinct (Lawless, 1984), and were described as 'side-bands' of capsaicin's pungency (Lawless and Stevens, 1988), the indirect evidence that capsaicin might stimulate taste neurons attracted little attention. At the same time, capsaicin's identity as a non-gustatory stimulus was reinforced by studies that evaluated its ability to mask taste (Lawless and Stevens, 1984; Lawless *et al.*, 1985; Cowart, 1987) and for its burn to be masked in turn by taste (Stevens and Lawless, 1986). Somewhat later, capsaicin's ability to desensitize pain fibers led to studies of cross-desensitization that were designed to assess possible chemesthetic (burning, stinging) components of the 'tastes' of some common gustatory stimuli (e.g. acids and bitter agents). The first of those studies found a strong effect of capsaicin desensitization on the burning and stinging produced by these stimuli, and only a small effect on the saltiness of NaCl and sourness of citric acid. The latter

effects were attributed to a failure to completely discriminate sensations of taste and irritation (Gilmore and Green, 1993). Karrer and Bartoshuk (Karrer and Bartoshuk, 1995) later found significant reductions in the bitterness of quinine hydrochloride (QHCl) and PROP (6-*n*-propyl-thiouracil) as well as in the sourness of citric acid, and also speculated that the effect was due chiefly to desensitization of the chemesthetic component of these tastes. However, Karrer and Bartoshuk noted that in some cases taste did not recover as quickly as chemesthesis, which left open the possibility that capsaicin had a direct desensitizing effect on taste. This possibility was consistent with a prior finding that rats that had been systemically desensitized to capsaicin had higher rejection thresholds to quinine than did non-desensitized control animals (Silver *et al.*, 1985). Most recently however, Simons *et al*. (Simons *et al*., 2002) reported finding no effect of capsaicin desensitization on perception of quinine on the tongue tip.

During preliminary testing for another study with capsaicin, a member of our laboratory reported that capsaicin tasted bitter on the back of the tongue. After this observation was confirmed in informal tests on other individuals in the lab, we undertook the present study to quantify capsaicin's gustatory effects on different parts of the tongue. Menthol, which is a sensory irritant as well as an artificial cooling agent (Green, 1992; Cliff and Green, 1994, 1996; Green and McAuliffe, 2000; Dessirier *et al.*, 2001b), was included as a second sensory irritant that had the potential to evoke taste sensations. Although never studied psychophysically as a gustatory stimulus, electrophysiological experiments with menthol had indicated that it briefly excites the rodent chorda tympani nerve before eventually suppressing it (Hellekant, 1969; Lundy and Contreras, 1993). It was therefore possible that capsaicin and menthol would both stimulate taste in humans, but via different gustatory nerves. After the first experiment indicated that both chemicals produced bitterness predominantly on the back of the tongue, a second experiment in which capsaicin was presented with other taste stimuli was run to rule out the possibility that bitterness ratings resulted from a qualitative confusion between bitterness and burning. Use of capsaicin in the second experiment also offered an opportunity to re-visit the question of whether sucrose and capsaicin have mutually inhibitory effects (Szolcsanyi, 1977; Lawless and Stevens, 1984, 1988; Lawless *et al.*, 1985; Cowart, 1987; Prescott *et al.*, 1993; Prescott and Stevenson, 1995).

## Materials and methods

### Subjects

A total of 56 adults (36 females and 20 males) between the ages of 18 and 45 years old participated in an initial screening session to determine eligibility for inclusion in two experiments (see Screening and Practice session section). Of the 56, 15 (11 females and four males) served in experiment 1, and 16 (nine females and seven males) served in experiment 2. All were recruited from public postings on and near the Yale University campus, and were paid for their participation.

### Stimuli

The same set of chemical stimuli was used in experiments 1 and 2, and all stimuli were applied via cotton-tipped swabs that were gently stroked onto areas of comparable size on the front, side and back of the tongue. In the Screening and Practice session capsaicin was applied in concentrations of 100 or 320 μM (98%, Aldrich, Milwaukee, WI), L-menthol in concentrations of 100 or 320 mM (99%, Haarman & Reimer, Teterboro, NJ). These concentrations are nominal values because the relative insolubility of capsaicin and menthol in water necessitated preparing the stimuli in ethanol solutions. To eliminate ethanol as a contaminating irritant during testing, the cotton swabs were dipped in the test solution prior to an experimental session and allowed to dry. The swabs were then tightly wrapped in plastic to prevent further loss of stimulus. The latter procedure was particularly critical for menthol because of its higher volatility than capsaicin. Just before application to the tongue, the swabs were rewetted by dipping them in deionized $H_2O$.

Four taste stimuli were used to give subjects practice in identifying and rating tastes prior to rating the capsaicin and menthol stimuli in both the Screening and Practice session, and the two main experiments. The stimuli used were 0.5 M sucrose, 0.5 M NaCl, 0.025 M citric acid and 1.0 mM quinine, all of which were prepared and presented in solution with deionized $H_2O$.

### Screening and practice session

It was recognized during preliminary testing that not everyone would perceive bitterness from capsaicin. Because we wished to study the characteristics of this bitterness, only individuals who reported at least weak bitterness from capsaicin applied to the circumvallate papillae were included in the study. In addition, because of differences in lingual anatomy and because the sensitivity of the gag reflex varies among individuals, the circumvallate papillae cannot be accessed reliably for testing in everyone. A two-step screening process was therefore instituted in which the experimenter determined if the circumvallate papillae could be visualized and stimulated with a cotton swab without inducing gagging. Individuals who passed the screening then received a series of taste stimuli followed by menthol and capsaicin. The four taste stimuli were presented once each to the front, side and back of the tongue in one of three pseudo-random orders. After each application, the subject rated the perceived intensity of sweetness, sourness, saltiness, bitterness, burning and coolness using the Labeled Magnitude Scale (LMS) (Green *et al.*, 1993, 1996), with 'strongest imaginable sensation of any kind' at the top of the scale. Before they

received the test stimuli, subjects were read instructions about how to use the LMS and were given practice with the scale by rating the intensity of 15 common oral sensations (e.g. the sweetness of cotton candy; the feel of a pill on the tongue) which they were asked to imagine. Ratings were made by using a mouse to move a cursor to the appropriate point on the LMS. For the actual stimuli, the six sensation qualities were rated sequentially on separate computer screens, always in the same order. After completing the ratings for a given stimulus, the subject rinsed three times with 37°C deionized $H_2O$, then received the next stimulus.

As part of the human subjects consent procedure, participants were told what kinds of taste stimuli they might receive, including capsaicin. However, to encourage a full qualitative analysis of the sensations, and to avoid any tendency for subjects to base ratings on the presumed identity of the stimulus (e.g. categorizing citric acid as sour only, or menthol as cool only), they were also told that the stimuli could be presented by themselves or in mixtures.

After the taste ratings were completed the experimenter applied 100 mM menthol to one side of the tongue tip. The subject was asked to rate the resulting sensations once per minute over a 5 min period, with the first rating coming 20 s after the stimulus was presented. The 20 s delay allowed the relatively slow-growing menthol sensation to reach a perceptible level. If at the end of the response period the subject had failed to rate the sensation as ≥ weak on at least one of the six qualitative dimensions (sweet, salty, sour, bitter, burning, coolness), they were allowed to rinse and then were given the 320 mM menthol solution on the opposite side of the tongue. The same procedure was followed for capsaicin, except that testing began on the right circumvallate papillae with the 100 μM solution. Failure to reach the criterion response there resulted in application of the 320 μM solution on the opposing circumvallate papillae All subjects reported ≥ weak sensations of one kind or another from one of the two concentrations of both chemicals. However, only those subjects who also reported ≥ weak bitterness from capsaicin in the circumvallate region were invited to return for the main experiment, and were then tested with the concentration at which they met the criterion.

## Experimental procedure

### *Experiment 1: capsaicin and menthol taste and irritation*

The purpose of experiment 1 was to compare the perception of capsaicin and menthol on three areas of the tongue: the tongue tip (fungiform papillae region), the posteriolateral tongue (foliate papillae region), and the dorsal surface of the back of the tongue (circumvallate papillae). The tongue tip was stimulated over an area extending ~1–2 cm lateral to the midline along the tongue blade; an area of comparable size was swabbed in the circumvallate region on the right-rear side of the tongue. Because the foliate papillae were frequently difficult to identify, testing in this region was conducted along a 1–2 cm swath about three-quarters of the distance from the tip to the root of the tongue on the right side. Capsaicin, menthol and the four prototype taste stimuli were tested at all three sites.

Because capsaicin and menthol interact temporally with one another (Cliff and Green, 1996; Green and McAuliffe, 2000; Dessirier *et al*., 2001b), the two chemicals were tested in different sessions separated by at least 48 h. Subjects therefore served in four experimental sessions that yielded two replicate ratings for capsaicin and menthol and four replicate ratings for each taste stimulus at each site. The sessions began with presentation of the four taste stimuli, which were applied once each to the three sites in one of three pseudo-random orders. Applying the prototypical taste stimuli first gave subjects practice rating taste qualities before receiving capsaicin or menthol, and avoided the possibility of carry-over effects from the chemesthetic stimuli (e.g. desensitization) that might interfere with perception of the taste stimuli. As in the practice session, subjects were told that the stimuli could be applied singly or in mixtures, and the LMS was again used to rate the intensity of sweetness, saltiness, sourness, bitterness, burning and coolness on every trial. Also as in the practice session, capsaicin and menthol were rated six times at 1 min intervals beginning 20 s after stimulus application. The additional ratings provided data on changes in intensity, and possibly quality, over time. The order of spatial testing for capsaicin and menthol was counterbalanced across individuals.

### *Experiment 2: inhibition of capsaicin bitterness by sucrose*

The purpose of experiment 2 was to determine if sucrose would suppress the bitterness of capsaicin in the same way it suppresses the bitterness of prototypical bitter stimuli. Capsaicin was also presented with a bitter tastant (QHCl) to see if subjects are able to discriminate bitterness from burning. That is, adding QHCl should increase bitterness but not burning. Because capsaicin was reported to be equally (and minimally) bitter on the foliate region and the tongue tip, testing was limited to the tongue tip and circumvallate regions.

Testing was conducted on both sides of the tongue in an alternating sequence, e.g. left tongue tip, right circumvallate papillae, right tongue tip, left circumvallate papillae, and the four taste stimuli were applied first as before. Each taste stimulus was presented to one side of the front and one side of the back, in pseudo-random order. After the individual taste stimuli had been tested, capsaicin was applied with one of three 'mixture' stimuli: 0.5 M sucrose, 1.0 mM QHCl and deionized $H_2O$ (control). The sequence of testing with capsaicin was the same as with the taste stimuli, i.e. each of the four sites (left and right sides, front and back) was tested once during a session, yielding two replicate ratings for the tongue tip and circumvallate regions for each stimulus.

Because it was essential for the taste sensation of the mixed solutions to be present when the capsaicin sensation

(bitterness or burning) first appeared, the taste stimulus was swabbed onto the test site immediately before applying capsaicin, and then was also used to wet the capsaicin swab. Thus the tongue was exposed to the taste solution just prior to and during application of capsaicin. The first ratings of taste and burning were again made 20 s after capsaicin, and represented the peak sensation perceived during that interval. Ratings were collected at 1 min intervals out to only 3 min 20 s, which experiment 1 indicated was adequate time to capture the effects of stimulus and test site.

Subjects served in three experimental sessions, with a different mixture solution (sucrose, quinine or water) presented with capsaicin in each session. There were two different orders of solution presentation across sessions, with half of the subjects receiving one order and half the other order.

## Results

### Experiment 1: capsaicin and menthol taste and irritation

Capsaicin and menthol were both perceived to have a bitter taste when applied to specific areas of the tongue. Figure 1 shows the log-means of the intensity ratings for the four taste qualities, burning and cold that were given in the first rating interval (20 s after application). Individual subject means were converted to logarithms to normalize the data before statistical analysis (Green *et al.*, 1993). For both stimuli there was a clear trend for bitterness to be strongest in the circumvallate region, where it was rated at least 'moderate' in intensity. No other taste qualities were rated significantly stronger than 'barely detectable'. Cold sensations, which were reported only with menthol, also tended to be higher on the circumvallate papillae, whereas burning was rated stronger for both chemicals in the fungiform region.

Figures 2 and 3 display the time course of perceived bitterness (Figure 2) and burning sensation (Figure 3) throughout the 5 min 20 s observation period. Both figures show clear effects of tongue location and time. A four-factor repeated measures ANOVA (stimulus × site × time × sensation quality) confirmed there was a significant main effect of time [$F(5,70) = 78.15$, $P < 0.0001$], and a significant interaction among stimulus, site and sensation quality [$F(2,28) = 13.0$, $P < 0.0001$] confirmed the visual impression from Figures 2 and 3 that capsaicin evoked more bitterness in the circumvallate region but more burn in the fungiform region, whereas menthol evoked more bitterness in the circumvallate region but about equal (and small) amounts of burn at all three sites. A significant four-way interaction [$F(10,140) = 3.51$, $P < 0.0005$] indicated that these effects varied over time, being larger initially and declining as time progressed and sensations diminished in intensity. Contributing to the interaction with time, however, was the slower decay in burning from capsaicin in the fungiform region than elsewhere.

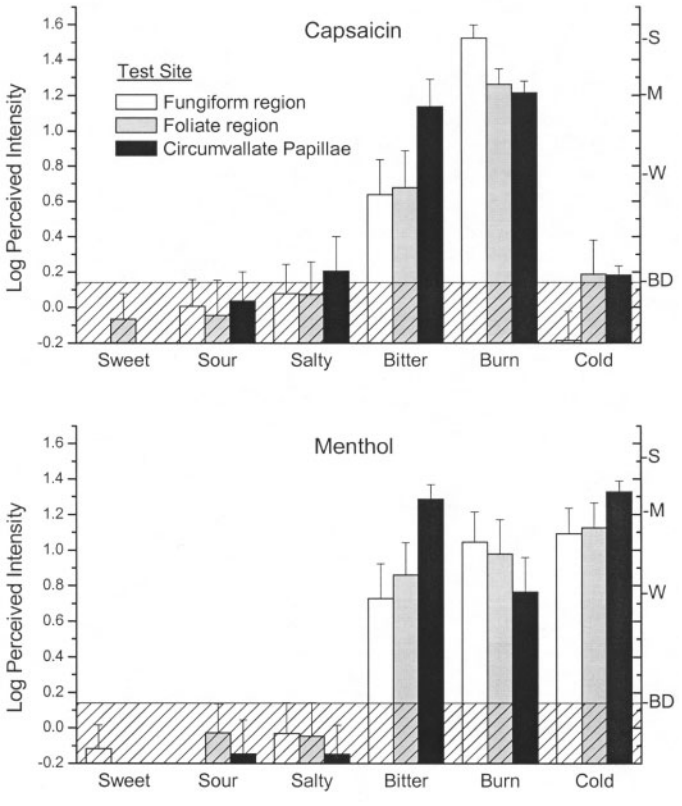


**Figure 1** Log means of perceived intensity of four taste qualities plus burning and cold rated 20 s after exposure are shown for capsaicin (top) and menthol (bottom) on three lingual sites. Bitterness ratings were highest for both stimuli in the circumvallate region (black bars), whereas burning tended to be strongest in the fungiform region (open bars). The foliate region (gray bars) generally yielded ratings similar to the fungiform region. The letters on the right *y*-axis indicate the locations of semantic descriptors on the LMS: BD = barely detectable; W = weak; M = moderate; S = strong. The hatched area indicates sensations that were judged less than 'barely detectable', and thus can be considered very near the threshold for detection. Vertical bars denote standard errors of the means (SEMs).

With respect to bitterness (Figure 2), the fungiform and foliate regions were similarly low in responsiveness; *post hoc* statistical tests revealed no significant differences between bitterness ratings for those two sites at any time point (Tukey HSD, all $P$s $> 0.05$). In contrast, the circumvallate region yielded significantly higher bitterness ratings for both chemicals over the first two ratings (Tukey HSD, $P < 0.05$). Bitterness from capsaicin was initially rated 3.2 times stronger in the circumvallate region than in the fungiform region, compared with 3.6 times stronger in the circumvallate region for menthol.

Burning sensations were not different across sites for menthol at any time, and were uniformly weak. As noted above, capsaicin induced stronger burning sensations in the fungiform region than in the circumvallate region. On average, the initial burn rating was exactly twice as high on the circumvallate papillae than in the foliate region (corresponding semantically to 'strong' versus 'moderate'

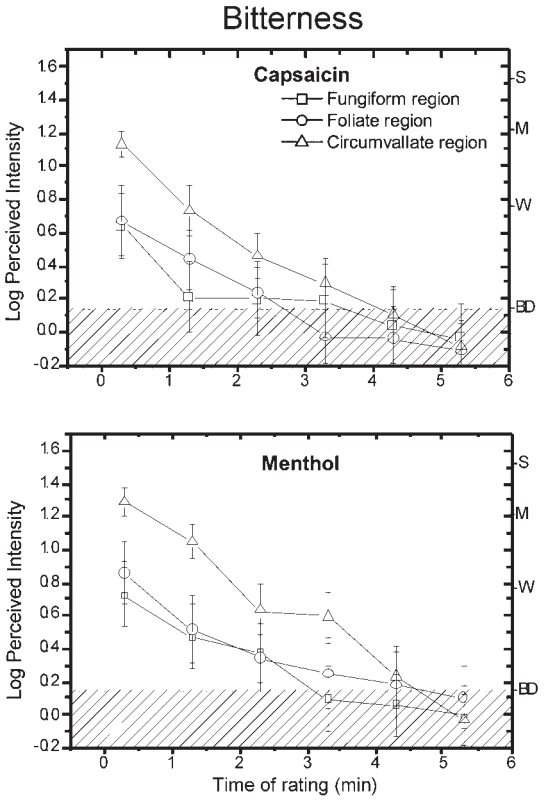


**Figure 2** The decay in perceived bitterness over time is shown for the first 5 min 20 s after exposure on the three areas of the tongue. The relative intensities of bitterness across sites, and the rate of decline over time, were similar for capsaicin (top) and menthol (bottom). The data points at 20 s correspond to the bitterness ratings shown in Figure 1. Vertical bars represent SEMs.

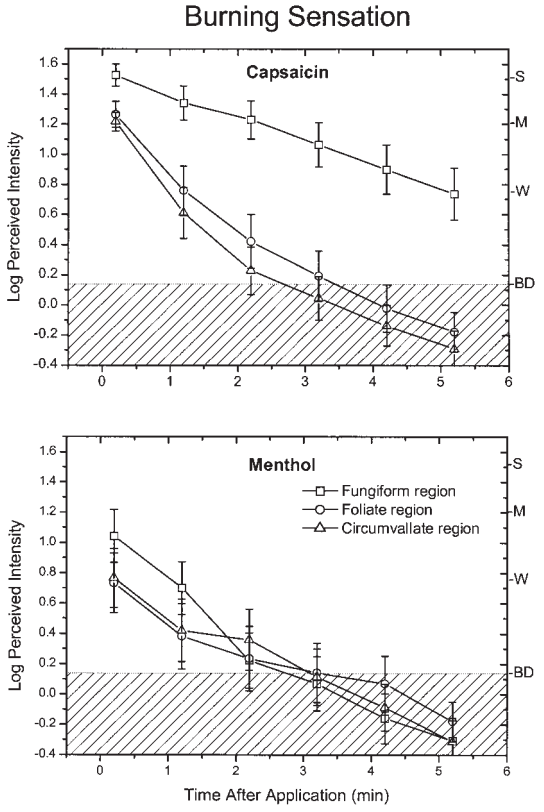


**Figure 3** Same as Figure 2, but for ratings of perceived burning sensation. At the concentrations tested, capsaicin produced much stronger burning than menthol at all three sites, with the fungiform region yielding significantly higher and more prolonged ratings than the other two areas. Vertical bars represent SEMs.

intensities), and the slower rate of decline in sensation on the back of the tongue caused this difference to grow proportionally larger over time.

Figure 4 displays the ratings for the four prototype taste stimuli. Taste responsiveness did not vary appreciably among the three sites. A three-factor (stimulus × site × quality) repeated measures ANOVA indicated that there was a significant main effect of stimulus [$F(3,42) = 5.9$, $P < 0.0001$] as well as interactions between stimulus and quality [$F(9,126) = 45.3$, $P < 0.0001$], and among stimulus, site and quality [$F(18,252) = 2.0$, $P < 0.05$]. *Post hoc* tests showed that the three-way interaction resulted from differences in the intensities of weak 'side tastes' at different sites rather than to differences in the principal taste evoked by each stimulus. Thus unlike the much stronger bitterness of capsaicin and menthol in the circumvallate region, a slight tendency for the bitterness of QHCl to be higher in the same region was not significant.

### Experiment 2: inhibition of capsaicin bitterness by sucrose

The effects of adding sucrose or quinine on perception of bitterness and burning from capsaicin are shown in Figures 5 and 6. The bitterness of capsaicin in deionized $H_2O$, which was similar to that of experiment 1, was strongly suppressed by sucrose (Figure 5, top). Compared to when capsaicin was tested alone, sucrose lowered average bitterness ratings by 87% in the first rating period. Sucrose was less effective in blocking the much weaker bitterness reported in the fungiform region; except for the first rating, bitterness was rated nearly the same with and without sucrose.

As expected, QHCl amplified bitterness ratings in both regions. The enhancement of bitterness was substantially larger in the fungiform region, where capsaicin by itself was only weakly bitter. Adding QHCl to capsaicin at that site increased bitterness by a factor of 3.5 to 1, compared with 1.6 to 1 in the circumvallate region.

A three-factor repeated measures ANOVA (stimulus × site × time) was conducted on the bitter taste ratings. All main effects and interactions were significant ($P < 0.05$). The three-way interaction among stimulus, site and time [$F(6,90) = 3.5$, $P < 0.005$] confirmed the very different effect of sucrose on capsaicin bitterness between the two sites. Tukey HSD tests ($P < 0.05$) showed that in the circumvallate region bitterness was significantly less at all time intervals with sucrose, whereas on the tongue tip sucrose had no

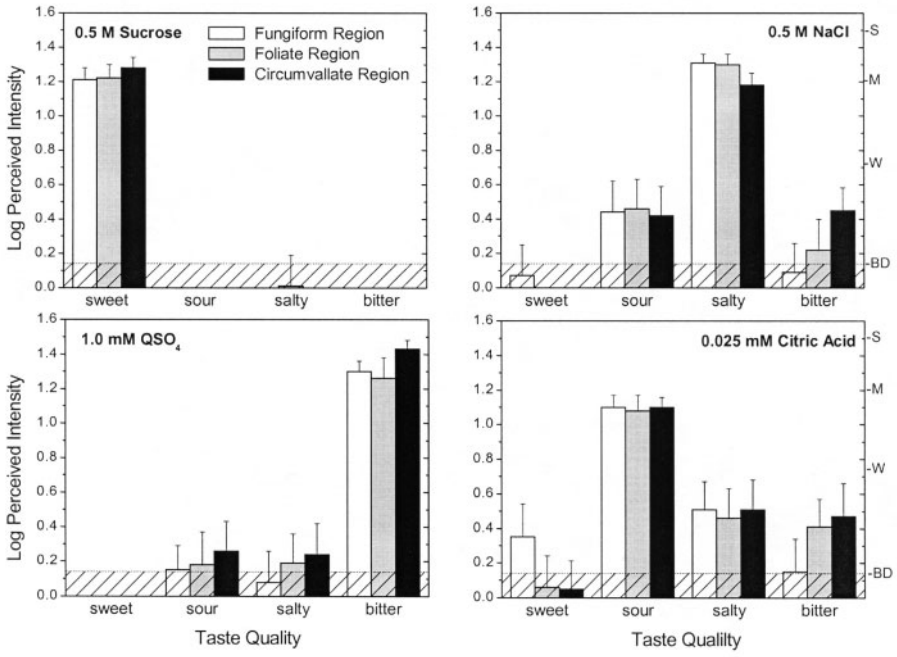


**Figure 4** Log means of perceived intensity for the four taste stimulus prototypes that were used to assess taste responsiveness at each of the three lingual test sites. Vertical bars represent SEMs.

significant effect on bitterness at any time. In addition, in the circumvallate region adding QHCl to capsaicin produced significantly higher bitterness ratings only over the last two ratings, whereas bitterness was higher on the tongue tip during only the first two ratings. The latter two outcomes appear from Figure 5 to be attributable to higher initial bitterness ratings for capsaicin in $H_2O$ in the circumvallate region and a more rapid decay in QHCl bitterness on the tongue tip. A separate three-factor repeated measures ANOVA on the burn ratings showed that there were main effects of stimulus [$F(1,15) = 30.4$, $P < 0.0001$], site [$F(1,15) = 34.6$, $P < 0.0001$] and time [$F(3,45) = 56.6$, $P < 0.0001$], but no significant interactions. The effect of stimulus, together with the absence of a significant interaction between stimulus and site, is the most notable outcome of the analysis, as it indicates that sucrose significantly reduced burn ratings at both sites (Figure 6). What appears to be a proportionally greater reduction in burn ratings in the circumvallate region was accompanied by a higher variance in the intensity ratings for that site.

Although sucrose significantly reduced burning sensations as well as bitterness in the circumvallate region, the reduction in burning was much less than the reduction in bitterness. Measured from their highest (initial) intensity levels, sucrose reduced the intensity of burning by 49%, compared with the 87% reduction in bitterness.

To determine if there was a reciprocal effect of capsaicin on the sweetness of sucrose, separate *t*-tests for dependent means were conducted on the sweetness intensity ratings when sucrose was presented alone in the taste portion of the experiment versus the first rating when it was presented with capsaicin. Although sweetness was lower at both sites when capsaicin was present (Figure 7), the reduction was significant only in the fungiform region ($t_{15} = 3.0$, $P < 0.01$).

## Discussion

The present results show that neither capsaicin nor menthol can be assumed to stimulate only somatosensory receptors in the oral cavity. Indeed, given capsaicin's pre-eminence as the prototypical chemesthetic stimulus, the present results raise the question whether any chemesthetic stimuli exist that do not also stimulate the gustatory system. It is likely that sensory irritants with chemical structures similar to capsaicin evoke a bitter taste [e.g. piperine (Lawless, 1984)], and the other two most frequently studied oral chemesthetic stimuli, ethanol and $CO_2$, have long been known to stimulate gustatory nerves in animals (Diamant *et al.*, 1963; Hellekant, 1965a,b; Kawamura and Adachi, 1967; Komai *et al.*, 1994), and to produce weak tastes in humans (Settle, 1979; Cowart, 1998). The sensory effects of capsaicin and menthol may be most similar to nicotine, which has been studied both as a sensory irritant (Jancso *et al.*, 1961; Jarvik and Assil, 1988; Liu and Simon, 1996, 1997; Dessirier *et al.*, 1997, 1999, 2000) and as a bitter tastant (Koyama and Kurihara, 1972; Kumar *et al.*, 1983; Liu and Simon, 1998; Ming *et al.*, 1999; Scott *et al.*, 1999; Katz *et al.*, 2002). Conversely, many prototypical taste stimuli have been shown to produce sensory irritation (Abrahams *et al.*, 1937; Holway and Hurvich, 1937; Green and Gelhard, 1989; Gilmore and Green, 1993). Thus it is best to avoid categor-

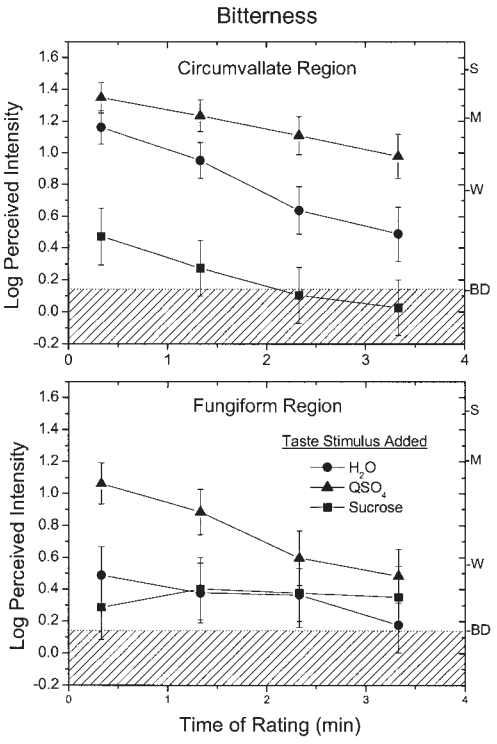


**Figure 5** Log means of perceived intensity of bitterness from capsaicin as a function of time in the circumvallate region (top) or in the fungiform region (bottom) of the tongue when capsaicin was presented with $H_2O$ (●), with $QSO_4$ (▲), or with sucrose (■). Vertical bars represent SEMs.

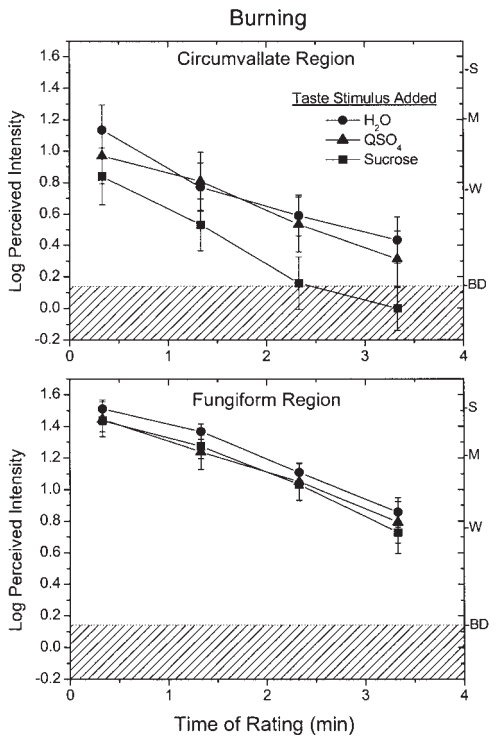


**Figure 6** Same as Figure 5, except that the dependent variable is the perceived intensity of burning sensation from capsaicin.

izing chemicals as strictly gustatory (e.g. 'tastants') or chemesthetic (e.g. 'irritants'), and instead describe them instead as principally chemesthetic or principally gustatory.

### Possible mechanisms of irritant bitter taste

The simplest explanation for the bitter taste from capsaicin and menthol is that both chemicals stimulate gustatory neurons that normally respond to bitter tasting substances. Evidence that the two chemicals stimulate only a subset of neurons that contribute to bitterness comes both from the marked individual differences in capsaicin bitterness, and from the large difference in bitterness ratings between the front and back of the tongue. As noted in the Materials and methods section, only 55% (31 of 56) of the people we screened reported at least weak bitterness from capsaicin in the circumvallate region. Although capsaicin 'non-tasters' were not thoroughly studied, all rated QHCl as bitter, and so appeared to have normal bitter perception. A reasonable hypothesis is that capsaicin non-tasters lack a particular type of gustatory neuron that can be stimulated by capsaicin as well as by more typical bitter substances.

Although it is possible that capsaicin stimulates gustatory neurons via a nonspecific biophysical effect (Feigin *et al.*, 1995), a more parsimonious explanation is that it acts via the vanilloid receptor VR1 (Caterina *et al.*, 1997; Tominaga *et al.*, 1998; Gunthorpe *et al.*, 2002). A nonspecific effect on

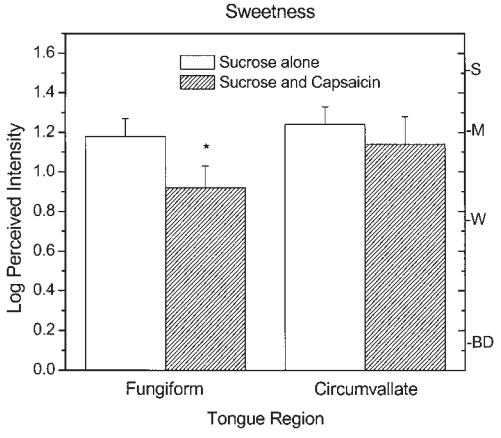


**Figure 7** Log perceived intensity of sweetness from 0.5 M sucrose either alone (open bars) or with capsaicin (hatched bars) on the fungiform and circumvallate regions of the tongue. Vertical bars represent SEMs; the asterisk indicates a significant difference between mean intensity ratings with and without capsaicin ($P < 0.001$).

neuronal membranes would be expected to occur with equal frequency across individuals, and probably between gustatory nerves, whereas a more specific excitatory effect would be vulnerable to variations in genetic expression of a particular molecular receptor or ion channel. Although VR1 has not yet been reported in association with gustatory

neurons or taste buds of the chorda tympani nerve, evidence of its presence in rat circumvallate papillae has been reported (Liu and Simon, 2001). However, VR1 in circumvallate papillae could be associated with somatosensory rather than gustatory neurons of the glossopharyngeal nerve, and in any case, its presence would not explain the bitterness of menthol, for which another receptor was recently identified [CMR1 (McKemy *et al.*, 2002); TRPM8 (Peier *et al.*, 2002)]. The menthol receptor and VR1 do, however, belong to the same family of transient receptor potential (TRP) ion channels, which introduces the possibility that some gustatory neurons might express both types of TRPs. Evidence of multiple, related receptor types, including ion channels gated by capsaicin, has been reported for rat trigeminal ganglion neurons (Liu and Simon, 2000), and mouse trigeminal ganglion neurons were recently reported that responded to menthol and cold as well as to capsaicin (Viana *et al.*, 2002). The latter finding indicates that it is possible for the same neuron to express capsaicin and menthol receptors, and may account for the cross-desensitization between menthol and capsaicin on the tongue that has been shown psychophysically (Cliff and Green, 1996; Green and McAuliffe, 2000).

#### Regional and individual differences in bitterness and burn

The differences in bitterness and burning sensations among the three lingual sites imply that substantial differences exist in the way chemesthetic stimuli are sensed on the front and back of the tongue. It is unlikely the stronger bitterness from menthol and capsaicin was due to a generally higher density of innervation of circumvallate papillae by gustatory fibers that respond to bitter substances. Consistent with earlier findings (Collings, 1974), QHCl was not rated significantly more bitter in the circumvallate region than in the foliate and fungiform regions. The alternative hypothesis is that taste receptor cells innervated by the glossopharyngeal nerve express different types and combinations of bitter receptors than those innervated by the chorda tympani nerve (Adler *et al.*, 2000; Chandrashekar *et al.*, 2000), including some that can be stimulated by capsaicin and menthol.

The equivocal suppression of capsaicin bitterness by sucrose on the tongue tip (experiment 2) suggests that bitterness ratings in that region may have arisen more from a response bias or qualitative confusion than from a true gustatory effect. That is, when asked to rate taste sensations from capsaicin, subjects may attribute a degree of bitterness to the burn because they consider burning sensations to be more similar in quality to bitterness than to sweetness, sourness or saltiness. One might argue that bitterness cannot be confused with burning because it lacks a 'tactile' quality, but confusion in the opposite direction may be more likely, i.e. subjects may find it difficult to rule out bitterness in the presence of burning. If a qualitative confusion did play a role, it is possible the human chorda tympani nerve lacks capsaicin-sensitive neurons altogether (Liu and Simon, 2001). The absence of such neurons might explain why bitterness was not reported in the vast majority of previous studies in which capsaicin was applied only to the tongue tip, or even when the entire anterior tongue was bathed in capsaicin during sip-and-spit procedures.

Burning sensations showed the opposite spatial pattern, with stronger burning reported from capsaicin on the tip of the tongue, along with the marginally stronger burning from menthol at the same site. Apparently, both chemicals stimulate nociceptors of the trigeminal nerve more strongly than they stimulate nociceptors of the glossopharyngeal nerve. The strong burning sensations on the front of the tongue are consistent with the abundant innervation of fungiform papillae by the trigeminal nerve, where in rodents the majority of nerve endings belong to the trigeminal nerve rather than to the chorda tympani nerve (Farbman and Hellekant, 1978; Whitehead *et al.*, 1985), and where somatosensory neurons appear earlier in development than do gustatory fibers (Whitehead and Kachele, 1994). Neurons that contain substance-P (SP), and thus are assumed to be nociceptors, are also found in abundance in and around taste buds of the circumvallate papillae (Finger, 1986; Kinnman and Aldskogius, 1991; Finger and Böttger, 1993), but a recent study of human circumvallate tissue indicates that as many as 98% of SP-containing fibers terminate in the connective tissue at the base of the papillae rather than in more superficial layers (Kusakabe *et al.*, 1998). Deeper-lying receptors would be expected to result in a lesser sensory response for a given stimulus and concentration, as the number of molecules that are able to reach the receptors would be smaller.

In contrast to bitterness and burning, menthol's coolness was perceived similarly on the front, side and tip of the tongue, which is consistent with prior evidence that sensitivity to physical cooling is relatively uniform in the mouth (Green and Gelhard, 1987). The spatial distribution of cold fibers in the oral cavity, which do not contain SP, is unknown, but the present data together with the data on cold sensitivity suggest that cold fibers from the trigeminal and glossopharyngeal nerves innervate the front and back of the tongue in similar densities and perhaps at similar depths in the mucosa.

Because we did not specifically screen for bitterness from menthol we do not know whether more people perceive menthol to be bitter than perceive capsaicin to be bitter. Results from a previous study in this laboratory indicated there were large individual differences in perception of menthol bitterness when solutions were sipped and held in the mouth rather than swabbed onto small areas of the tongue (our unpublished data), but in the present experiment all subjects who perceived bitterness from capsaicin also reported bitterness from menthol. An experiment is needed in which both stimuli, as well as other irritants (e.g. nicotine, ethanol, piperine), are applied to the front and back of the tongue in a large group of subjects to determine

the extent to which the bitterness of chemesthetic stimuli co-varies across people and between the innervation fields of the chorda tympani and glossopharyngeal nerves.

### The effect of capsaicin on the sweetness of sucrose

Several studies have investigated whether capsaicin can mask or suppress tastes (Szolcsanyi, 1977; Lawless and Stevens, 1984; Lawless *et al*., 1985; Cowart, 1987; Prescott *et al*., 1993; Prescott and Stevenson, 1995; Simons *et al*., 2002). Although differences in methodology make comparisons among results difficult, the most consistent finding has been a small reduction in sweetness. The present results support that conclusion, particularly in the fungiform region, where most measurements have been made. Because bitterness and sweetness tend to suppress one another (Kroeze, 1980; Lawless, 1982), the discovery that capsaicin can induce bitterness offered a possible explanation for reductions in sweetness by capsaicin. However, data from experiment 2 indicate that suppression of sweetness by bitterness plays little or no role. Despite the stronger bitterness from capsaicin in the circumvallate papillae, sucrose was rated significantly weaker in the presence of capsaicin only in the fungiform region, where burning was strong and bitterness was weak. This result does not entirely rule out an effect of capsaicin's bitterness on sweetness, but it does indicate that masking by sensory irritation, or by some indirect effect of sensory irritation [e.g. release of cytokines from peptidergic neurons in and around taste papillae (Wang *et al*., 1995)], is likely to be a bigger factor.

### The effect of sucrose on the burn of capsaicin

The opposite effect, namely a reduction in capsaicin's burning sensation by sucrose (Stevens and Lawless, 1986), may be a byproduct of sucrose's ability to suppress bitterness. To the extent that perceptual discrimination of bitterness and burn is imperfect, a large reduction in bitterness should result in lower ratings of burn as well. It is conceivable that the nearly 50% reduction in the relatively weak burning sensation from capsaicin in the circumvallate region might have been largely caused by the very strong suppression of bitterness (87%) by sucrose at the same site. This explanation is consistent with the lesser effect of sucrose on burning sensations in the fungiform region, where the much weaker bitterness reported in experiment 2 was not reduced significantly by sucrose. A difficulty with this explanation is that adding QHCl to capsaicin increased perceived bitterness without also increasing ratings of burning (Figures 5 and 6). In fact, there was an overall trend toward lower burning ratings when QHCl was added, with the effect in the fungiform region equaling that of sucrose. Such an outcome is more in accord with the hypothesis that the gustatory system exerts a tonic inhibitory pressure on oral somatosensation and pain (Tie *et al*., 1999). Any tonic inhibitory effect might be augmented by phasic excitation of the gustatory system, and perhaps more strongly by some subsets of gustatory neurons (e.g. those involved in encoding sweetness) than by others.

## Summary and conclusions

In addition to its well known somatosensory quality of burning, capsaicin was shown to be capable of producing significant bitterness, particularly in the circumvallate region of the tongue. Menthol, another commonly studied chemesthetic stimulus, evoked similar amounts of bitterness. Because sucrose significantly suppressed the moderate bitterness reported for capsaicin in the circumvallate region, we conclude that capsaicin is a gustatory stimulus for the glossopharyngeal nerve. The equivocal effect of sucrose on the weaker bitterness reported on the front of the tongue does not permit the same conclusion with respect to the chorda tympani nerve. The most straightforward explanation of how capsaicin and menthol stimulate bitterness via the glossopharyngeal nerve is that some gustatory receptor cells in circumvallate papillae that are sensitive to bitter-tasting substances also express receptors that respond to capsaicin and menthol. Because only 55% of the individuals who were tested reported at least weak bitterness from capsaicin in the circumvallate region, our results predict that these receptors are differentially expressed across people. In future studies we will investigate the possible association of other bitter tasting compounds to the bitterness from capsaicin and menthol, and determine whether pre-exposure to capsaicin (or menthol) alters the bitterness of these compounds in ways that could be predicted from cross-desensitization of specific receptors and neurons.

## Acknowledgement

This research was supported in part by a grant from the National Institutes of Health, RO1 DC05002.

## References

**Abrahams, H., Krakauer, D.** and **Dallenbach, K.M.** (1937) *Gustatory adaptation to salt*. Am. J. Psychol., 49, 462–469.

**Adler, E., Hoon, M.A., Mueller, K.L., Chandrashekar J., Ryba, N.J** and **Zuker, C.S.** (2000) *A novel family of mammalian taste receptors*. Cell, 100, 693–702.

**Buck, S.H.** and **Burks, T.F.** (1986) *The neuropharmacology of capsaicin: review of some recent observations*. Pharmacol. Rev., 38, 179–226.

**Caterina, M.J., Schumacher, M.A., Tominaga, M., Rosen, T.A., Levine, J.D.** and **Julius, D.** (1997) *The capsaicin receptor: a heat-activated ion channel in the pain pathway*. Nature, 389, 816–824.

**Chandrashekar, J., Mueller, K.L., Hoon, M.A., Adler, E., Feng, L., Guo, W., Zuker, C.S.** and **Ryba, N.J.** (2000) *T2Rs function as bitter taste receptors*. Cell, 100, 703–711.

**Cliff, M.A.** and **Green, B.G.** (1994) *Sensory irritation and coolness produced by menthol: evidence for selective desensitization of irritation*. Physiol. Behav., 56, 1021–1029.

**Cliff, M.A.** and **Green, B.G.** (1996) *Sensitization and desensitization to menthol and capsaicin in the oral cavity: interactions and individual differences.* Physiol. Behav., 59, 487–494.

**Collings, V.B.** (1974) *Human taste response as a function of locus of stimulation on the tongue and soft palate*. Percept. Psychophys., 16, 169–174.

**Cowart, B.J.** (1987) *Oral chemical irritation: does it reduce perceived taste intensity?* Chem. Senses, 12, 467–479.

**Cowart, B.J.** (1998) *The addition of $CO_2$ to traditional taste solutions alters taste quality*. Chem. Senses, 23, 397–402.

**Dessirier, J.M., O'Mahony, M.** and **Carstens, E.** (1997) *Oral irritant effects of nicotine: psychophysical evidence for decreased sensation following repeated application and lack of cross-desensitization to capsaicin*. Chem. Senses, 22, 483–492.

**Dessirier, J.M., Nguyen, N., Sieffermann, J.M. Carstens, E.** and **O'Mahony, M.** (1999) *Oral irritant properties of piperine and nicotine: psychophysical evidence for asymmetrical desensitization effects*. Chem. Senses, 24, 405–413.

**Dessirier, J.M., Simons, C.T., Sudo, M., Sudo, S.** and **Carstens, E.** (2000) *Sensitization, desensitization and stimulus-induced recovery of trigeminal neuronal responses to oral capsaicin and nicotine*. J. Neurophysiol., 84, 1851–1862.

**Dessirier, J.M., Simons, C.T., O'Mahony, M.** and **Carstens, E.** (2001a) *The oral sensation of carbonated water: cross-desensitization by capsaicin and potentiation by amiloride*. Chem. Senses, 26, 639–643.

**Dessirier, J.M., O'Mahony, M.** and **Carstens, E.** (2001b) *Oral irritant properties of menthol: sensitizing and desensitizing effects of repeated application and cross-desensitization to nicotine*. Physiol. Behav., 73, 25–36.

**Diamant, H., Funakoshi, M., Strom, L.** and **Zotterman, Y.** (1963) *Electrophysiological studies on human taste nerves.* In Zotterman, Y. (ed.), Olfaction and Taste. Pergamon, Oxford, pp. 193–203.

**Farbman, A.I.** and **Hellekant, G.** (1978) *Quantitative analyses of the fiber population in rat chorda tympani nerves and fungiform papillae*. Am. J. Anat., 153, 509–552.

**Feigin, A.M., Aronov, E.V., Bryant, B.P., Teeter, J.H.** and **Brand J.G.** (1995) *Capsaicin and its analogs induce ion channels in planar lipid bilayers*. Neuroreport, 6, 2134–2136.

**Finger, T.E.** (1986) *Peptide immunohistochemistry demonstrates multiple classes of perigammal nerve fibers in the circumvallate papilla of the rat*. Chem. Senses, 11, 135–144.

**Finger, T.E.** and **Böttger, B.** (1993) *Peripheral peptidergic fibers of the trigeminal nerve in the olfactory bulb of the rat*. J. Comp. Neurol., 334, 117–124.

**Gilmore, M.M.** and **Green, B.G.** (1993) *Sensory irritation and taste produced by NaCl and citric acid: effects of capsaicin desensitization*. Chem. Senses, 18, 257–272.

**Green, B.G.** (1989) *Capsaicin sensitization and desensitization on the tongue produced by brief exposures to a low concentration*. Neurosci. Lett., 107, 173–178.

**Green, B.G.** (1992) *The sensory effects of l-menthol on human skin*. Somatosens. Mot. Res., 9, 235–244.

**Green, B.G.** and **Gelhard, B.** (1987) *Perception of temperature on oral and facial skin.* Somatosens. Mot. Res., 4, 191–200.

**Green, B.G.** and **Gelhard, B.** (1989) *Salt as an oral irritant*. Chem. Senses, 14, 259–271.

**Green, B.G.** and **McAuliffe, B.L.** (2000) *Menthol desensitization of capsaicin irritation. Evidence of a short-term anti-nociceptive effect.* [In Process Citation] Physiol. Behav., 68, 631–639.

**Green, B.G., Shaffer, G.S.** and **Gilmore, M.M.** (1993) *Derivation and evaluation of a semantic scale of oral sensation magnitude with apparent ratio properties*. Chem. Senses, 18, 683–702.

**Green, B.G., Dalton, P., Cowart, B.J., Shaffer, G., Rankin, K.** and **Higgins, J.** (1996) *Evaluating the 'Labeled Magnitude Scale' for measuring sensations of taste and smell*. Chem. Senses, 21, 323–334.

**Gunthorpe, M.J., Benham, C.D., Randall, A.** and **Davis, J.B.** (2002) *The diversity in the vanilloid (TRPV) receptor family of ion channels*. Trends Pharmacol. Sci., 23, 183–191.

**Hellekant, G.** (1965a) *Electrophysiological investigation of the gustatory effect of ethyl alcohol: I. The summated response of the chorda tympani in the cat, dog and rat.* Acta Physiol. Scand., 64, 392–397.

**Hellekant, G.** (1965b) *Electrophysiological investigation of the gustatory effect of ethyl alcohol: II. A single fiber analysis in the cat*. Acta Physiol. Scand., 64, 398–406.

**Hellekant, G.** (1969) *The effect of menthol on taste receptors*. Acta Physiol. Scand., 76, 361–368.

**Hettinger, T.P.** and **Frank, M.E.** (1992) *Information processing in mammalian gustatory systems.* Curr. Opin. Neurobiol., 2, 469–478.

**Holway, A.H.** and **Hurvich, L.M.** (1937) *Differential gustatory sensitivity to salt*. Am. J. Psychol., 48, 49–37.

**Jancso, N.** (1960) *Role of the nerve terminals in the mechanism of inflammatory reactions.* Bull. Millard Fillmore Hosp., 7, 53–77.

**Jancso, N., Jancso-Gabor, A.** and **Takats, I.** (1961) *Pain and inflammation induced by nicotine, acetylcholine and structurally related compounds and their prevention by desensitizing agents.* Acta Physiol. Acad. Sci. (Hung.), 19, 113–132.

**Jarvik, M.E.** and **Assil, K.M.** (1988) *Mecamylamine blocks the burning sensation of nicotine on the tongue*. Chem. Senses, 13, 213–217.

**Karrer, T.** and **Bartoshuk, L.** (1991) *Capsaicin desensitization and recovery on the human tongue*. Physiol. Behav., 49, 757–764.

**Karrer, T.** and **Bartoshuk, L.** (1995) *Effects of capsaicin desensitization on taste in humans*. Physiol. Behav., 57, 421–429.

**Katz, D.B., Simon, S.A.** and **Nicolelis, M.A.** (2002) *Taste-specific neuronal ensembles in the gustatory cortex of awake rats*. J. Neurosci., 22, 1850–1857.

**Kawamura, Y.** and **Adachi, A.** (1967) *Electrophysiological analysis of taste effectiveness of soda water and $CO_2$ gas*. In Hayashi, T. (ed.), Olfaction and Taste II. Pergamon, New York, pp. 431–437.

**Komai, M., Bryant, B., Takeda, T., Suzuki, H.** and **Kimura, S. (**1994) *The effect of carbonic anhydrase inhibitor, MK-927, on the response of the chorda tympani nerve to carbonated water.* In Kurikara, K., Suzuki, N. and Ogawa, H. (eds), Olfaction and Taste XI. Springer, Tokyo, pp. 92–99.

**Kinnman, E.** and **Aldskogius, H.** (1991) *The role of substance P and calcitonin gene-related peptide containing nerve fibers in maintaining fungiform taste buds in the rat after a chronic chorda tympani nerve injury*. Exp. Neurol., 113, 85–91.

**Koyama, N.** and **Kurihara, K.** (1972) *Mechanism of bitter taste reception: interaction of bitter compounds with monolayers of lipids from bovine circumvallate papillae*. Biochim. Biophys. Acta, 288, 22–26.

**Kroeze, J.H.** (1980) *Masking in two- and three-component taste mixtures.* In Van der Starre, H. (ed.), Olfaction and Taste VII. IRL Press, London, pp. 435–448.

**Kumar, R., Pratt, J.A.** and **Stolerman, I.P.** (1983) *Characteristics of conditioned taste aversion produced by nicotine in rats*. Br. J. Pharmacol., 79, 245–253.

**Kusakabe, T., Matsuda, H., Gono, Y., Furukawa, M., Hiruma, H., Kawakami, T., Tsukuda, M.** and **Takenaka, T.** (1998) *Immunohistochemical localisation of regulatory neuropeptides in human circumvallate papillae*. J. Anat., 192, 557–564.

**Lawless, H.T.** (1982) *Paradoxical adaptation to taste mixtures.* Physiol. Behav., 25, 149–152.

**Lawless, H.T.** (1984) *Oral chemical irritation: psychophysical properties.* Chem. Senses, 9, 143–157.

**Lawless, H.T.** and **Stevens, D.A.** (1984) *Effects of oral chemical irritation on taste.* Physiol. Behav., 32, 995–998.

**Lawless, H.T.** and **Stevens, D.A.** (1988) *Responses by humans to oral chemical irritants as a function of locus of stimulation.* Percept. Psychophys., 43, 72–78.

**Lawless, H.T., Rozin, P.** and **Shenker, J.** (1985) *Effects of oral capsaicin on gustatory, olfactory and irritant sensations and flavor identification in humans who regularly or rarely consume chili pepper.* Chem. Senses, 10, 579–589.

**Liu, L.** and **Simon, S.A.** (1996) *Capsaicin and nicotine both activate a subset of rat trigeminal ganglion neurons*. Am. J. Physiol., 270, C1807–C1814.

**Liu, L.** and **Simon, S.A.** (1997) *Capsazepine, a vanilloid receptor antagonist, inhibits nicotinic acetylcholine receptors in rat trigeminal ganglia*. Neurosci. Lett., 228, 29–32.

**Liu, L.** and **Simon, S.A.** (1998) *Responses of cultured rat trigeminal ganglion neurons to tastants*. Chem. Senses, 23, 125–130.

**Liu, L.** and **Simon, S.A.** (2000) *Capsaicin, acid and heat-evoked currents in rat trigeminal ganglion neurons: relationship to functional VR1 receptors*. Physiol. Behav., 69, 363–378.

**Liu, L.** and **Simon, S.A.** (2001) *Acidic stimuli activates two distinct pathways in taste receptor cells from rat fungiform papillae.* Brain Res., 923, 58–70.

**Liu, L., Wang, Y.** and **Simon, S.A.** (1996) *Capsaicin activated currents in rat dorsal root ganglion cells*. Pain, 64, 191–195.

**Lundy, R.F., Jr** and **Contreras, R.J.** (1993) *Taste prestimulation increases the chorda tympani nerve response to menthol*. Physiol. Behav., 54, 65–70.

**McKemy, D.D., Neuhausser, W.M.** and **Julius, D.** (2002) *Identification of a cold receptor reveals a general role for TRP channels in thermosensation*. Nature, 416, 52–58.

**Ming, D., Ninomiya, Y.** and **Margolskee, R.F.** (1999) *Blocking taste receptor activation of gustducin inhibits gustatory responses to bitter compounds*. Proc. Natl. Acad. Sci. USA, 96, 9903–9908.

**Peier, A.M., Moqrich, A., Hergarden, A.C., Reeve, A.J., Andersson, D.A., Story, G.M., Earley, T.J., Dragoni, I., McIntyre, P., Bevan, S.** and **Patapoutian, A.** (2002) *A TRP channel that senses cold stimuli and menthol*. Cell, 108, 705–715.

**Prescott, J.** and **Stevenson, R.J.** (1995) *Effects of oral chemical irritation on tastes and flavors in frequent and infrequent users of chili*. Physiol. Behav., 58, 1117–1127.

**Prescott, J., Allen, S.** and **Stephens, L.** (1993) *Interactions between oral chemical irritation, taste and temperature*. Chem. Senses, 18, 389–404.

**Scott, T.R., Giza, B.K.** and **Yan, J.** (1999) *Gustatory neural coding in the cortex of the alert cynomolgus macaque: the quality of bitterness*. J. Neurophysiol., 81, 60–71.

**Settle, R.G.** (1979) *Alcoholic's taste perception of alcohol: preliminary findings*. In Galanter, M. (ed.), Currents in Alcoholism, Vol. V: Biomedical Issues and Clinical Effects of Alcoholism. Grune & Stratton, New York, pp. 257–267.

**Silver, W.L., Mason, J.R., Marshall, D.A.** and **Maruniak, J.A.** (1985) *Rat trigeminal, olfactory and taste responses after capsaicin desensitization.* Brain Res., 333, 45–54.

**Simons, C.T., O'Mahony, O.** and **Carstens, E.** (2002) *Taste suppression following lingual capsaicin pre-treatment in humans.* Chem. Senses, 27, 353–365.

**Stevens, D.A.** and **Lawless, H.T.** (1986) *Putting out the fire: effects of tastants on oral chemical irritation.* Percept. Psychophys., 39, 346–350.

**Szallasi, A., Conte, B., Goso, C., Blumberg, P.M.** and **Manzini, S.** (1993) *Characterization of a peripheral vanilloid (capsaicin) receptor in the urinary bladder of the rat*. Life Sci., 52, PL221–PL226.

**Szolcsanyi, J.** (1977) *A pharmacological approach to elucidation of the role of different nerve fibres and receptor endings in mediation of pain*. J. Physiol. (Paris), 73, 251–259.

**Tie, K., Fast, K., Kveton, J.F., Cohen, Z., Duffy, V., Prudkin, J., Green, B.G.** and **Bartoshuk, L.M.** (1999) *Anesthesia of the chorda tympani nerve and the effect on pain.* Chem. Senses, 24, 609 **[**Abstract].

**Tominaga, M., Caterina, M.J., Malmberg, A.B., Rosen, T.A., Gilbert, H., Skinner, K., Raumann, B.E., Basbaum, A.I.** and **Julius, D.** (1998) *The cloned capsaicin receptor integrates multiple pain-producing stimuli*. Neuron, 21, 531–543.

**Viana, F., De La, P.E.** and **Belmonte, C.** (2002) *Specificity of cold thermotransduction is determined by differential ionic channel expression*. Nat. Neurosci., 5, 254–260.

**Wang, Y., Erickson, R.P.** and **Simon, S.A.** (1995) *Modulation of rat chorda tympani nerve activity by lingual nerve stimulation.* J. Neurosci., 73, 1468–1483.

**Whitehead, M.C.** and **Kachele, D.L.** (1994) *Development of fungiform papillae, taste buds, and their innervation in the hamster*. J. Comp. Neurol., 340, 515–530.

**Whitehead, M.C., Beeman, C.S.** and **Kinsella, B.A.** (1985) *Distribution of taste and general sensory nerve endings in fungiform papillae of the hamster*. Am. J. Anat., 173, 185–201.

*Accepted October 30, 2002*