Suppression of Central Taste Transmission by Oral Capsaicin

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Because intraoral capsaicin is reported to reduce the perceived intensity of certain taste qualities, we investigated whether it affects the central processing of gustatory information. The responses of gustatory neurons in the nucleus tractus solitarius (NTS) to tastant stimuli were recorded before and after lingual application of capsaicin in anesthetized rats. Thirty-four NTS units were characterized as responding best to sucrose (0.3 M), NaCl (0.1 M), citric acid (0.03 M), monosodium glutamate (0.2 M), or quinine (0.001 M). During lingual application of 330 μM capsaicin for 7 min, the firing rate increased for five units and decreased for four units; the remainder were unaffected. Immediately after capsaicin, responses to each tastant were in nearly all cases depressed (mean, 61.5% of control), followed by recovery in most cases. NTS tastant-evoked unit responses were unaffected by lingual application of vehicle (5% ethanol). Capsaicin elicited an equivalent reduction (to 64.5%) in tastant-evoked responses of nine additional NTS units recorded in rats with bilateral trigeminal ganglionectomy, arguing against a trigeminally mediated central effect. Furthermore, capsaicin elicited a puncate pattern of plasma extravasation in the tongue that matched the distribution of fungiform papillae. These results support a peripheral site of capsaicin suppression of taste possibly via direct or indirect effects on taste transduction or taste receptor cell excitability. The depressant effect of capsaicin on gustatory transmission might underlie its ability to reduce the perceived intensity of some taste qualities.

Key words: **capsaicin; rat; gustatory; nucleus of the solitary tract; trigeminal; taste; irritation**

Introduction

Integration of sensory information from separate modalities is an important process influencing perception and behavior. Sensations of taste, olfaction, and oral irritation contribute to food flavor, which significantly impacts food choice and nutritional status (Scott and Verhagen, 2000). Much of the integration of taste and smell appears to occur in the orbitofrontal cortex (Rolls and Baylis, 1994). Psychophysical evidence suggests that oral nociceptive input can suppress some taste qualities (Lawless and Stevens, 1984; Prescott and Stevenson, 1995; Simons et al., 2002a), but it is not clear whether this occurs at the cortical level (as flavor) or at earlier gustatory relays, as we have investigated presently.

Sapid tastants depolarize taste receptor cells in the tongue (Herness and Gilbertson, 1999) to excite primary gustatory neurons whose afferent fibers pass in the chorda tympani branch of the facial nerve to project to the rostral pole of the nucleus of the solitary tract (NTS) (Hamilton and Norgren, 1984; Hettinger and Frank, 1992), where the initial processing of taste quality and intensity occurs. Neurons responsive to both gustatory and lingual somatosensory stimuli have been identified in the NTS (Ogawa et al., 1984, 1988; Hayama et al., 1985; Travers and Norgren, 1995), but it is not known whether these neurons are modulated by noxious stimuli. Capsaicin, the pungent chemical in chili peppers, excites nociceptive trigeminal nerve endings in oral epithelia (Liu and Simon, 1996; Caterina et al., 1997) whose afferent fibers project via the lingual nerve to the brainstem trigeminal complex, most notably the subnucleus caudalis (Vc) (Sessle and Greenwood, 1976; Hu et al., 1981; Dubner and Bennett, 1983), which contains neurons responsive to oral chemical irritants (Carstens et al., 1998; Simons et al., 1999; Dessirier et al., 2000; Sudo et al., 2002). To investigate a possible effect of oral irritation on taste processing, we tested whether capsaicin reduced tastant-evoked responses of gustatory NTS neurons. Finding this to be the case, we also investigated whether the suppression occurs at a central or peripheral site. A substrate for central trigeminal modulation of gustatory processing in the NTS is supported by anatomical studies showing projections to the NTS from branches of trigeminal afferents (Jacquin et al., 1982; Whitehead and Frank, 1983; Hamilton and Norgren, 1984) as well as from neurons in Vc and paratrigeminal nucleus (Menétrey and Basbaum, 1987; Saxon and Hopkins, 1998). Some trigeminal fibers projecting to the NTS contain substance P (South and Ritter, 1986), which modulates NTS responses to NaCl (Davis and Smith, 1997). The possibility of a trigeminally mediated central effect was tested by determining whether capsaicin still depressed NTS tastant-evoked responses in animals with bilateral trigeminal ganglionectomy. Alternatively, a peripheral locus for capsaicin suppression of taste processing is supported by reports that oral capsaicin, or lingual nerve stimulation, depresses chorda tympani fiber responses to NaCl (Wang et al., 1995; Osada et al., 1997). As a potential peripheral substrate for trigeminal–gustatory interactions, we investigated whether capsaicin induces plasma extravasation in taste papillae. Portions of this work have been published previously in abstract form (Simons et al., 2001b; Carstens et al., 2002).

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Materials and Methods

Animals

A total of 73 adult male Sprague Dawley rats (Simonsen Inc., Gilroy, CA), weighing between 350 and 480 gm were used in these experiments. They were housed two per cage in a vivarium maintained on a 12 hr light/dark cycle at ~21°C. Food and water were available *ad libitum*. All procedures were in accordance with the National Institutes of Health animal welfare guide and were approved by the University of California Davis Animal Use and Care Advisory committee.

Electrophysiological experiments

Surgery. Animals were anesthetized with sodium pentobarbital (65 mg/kg, i.p.). Core body temperature was maintained at ~37°C by placing the animal on a heating pad. A midline incision was made over the trachea, and the hypoglossal nerve was cut bilaterally to prevent spontaneous tongue movement, followed by tracheotomy and cannulation of the jugular vein to allow constant infusion of pentobarbital (10 $mg \cdot kg^{-1} \cdot hr^{-1}$). The head was fixed in a stereotaxic frame using atraumatic earbars, and the transverse sinus was exposed and ligated bilaterally with silk suture. The cerebellum was then aspirated to expose the underlying medulla. The mouth was maintained in an open position to allow access to the oral cavity, which was wetted with distilled water to prevent desiccation.

For rats receiving bilateral trigeminal ganglionectomy ($n = 9$), the identical procedures were followed with the addition that the trigeminal ganglia were bilaterally exposed and sectioned with a microknife. The completeness of the ganglionectomy was verified by visual inspection. This procedure ensured complete blockade of input from the three branches of the trigeminal nerve bilaterally.

Recording and stimulation. A Teflon-insulated tungsten recording electrode (18–20 MΩ; Frederick Haer Company. Brunswick, ME) was advanced into the brainstem (2.7 mm anterior to obex; 1.8 mm lateral to midline) using a hydraulic microdrive (David Kopf Instruments, Tujunga, CA). Extracellular single-unit activity was amplified and displayed by conventional means and fed to a computer for analysis and storage. Recordings were made from gustatory neurons in the NTS with receptive fields anterior to the premolar eminence. Single units responsive to gustatory stimuli were routinely observed at depths ranging from ~700 to 1000 μm below the brainstem surface.

Gustatory NTS units were searched for using a taste mixture containing the following reagent-grade chemicals: sucrose (0.3 M; Mallinkrodt, Paris, KY), NaCl (0.1 M; Fisher Scientific, Fair Lawn, NJ), citric acid (0.03 M; Mallinkrodt), quinine HCl (0.01 M; Sigma, St. Louis, MO), and monosodium glutamate (MSG) (0.2 M; Sigma). Once a responsive unit was isolated, each of the five tastants was applied individually by hand-held syringe at a constant flow rate (~0.2 ml/sec) to the anterior lingual surface for 15 sec and left on for an additional 15 sec, followed immediately by a distilled water rinse (3 ml). Activity was recorded beginning 30 sec before the gustatory stimulus until 30 sec after stimulus cessation. Responses were quantified as the total number of impulses during the 30 sec stimulus period. Each tastant was applied at least two times to establish which one elicited the relatively largest response. The "best" tastant identified in this manner was then reapplied three times successively to establish response reproducibility. All solutions were delivered at room temperature to avoid any confounding effects of heating or cooling.

Those units exhibiting stable responses (within ±10% of mean response level to three applications) were then tested with capsaicin. Capsaicin [330 μM (100 ppm) in 5% ethanol; Sigma] was applied by hand-held syringe at a constant flow rate (~0.1 ml/sec) to the anterior lingual surface bilaterally for 7 min. We hypothesized that any depressant effect of capsaicin is mediated by activation of the trigeminal system, and therefore chose a concentration and duration of capsaicin application that is known to excite central trigeminal neurons (Dessirier et al., 2000). The 7 min stimulus duration was selected because most Vc neurons exhibited a plateau in maximal firing rate to intermittent or continual application of capsaicin by this time (Dessirier et al., 2000). Although the capsaicin concentration used presently exceeds levels (~1 μM) capable of activating vanilloid receptor-1 receptors expressed in trigeminal ganglion cells *in vitro* (Liu and Simon, 1996), the lingual epithelium represents a substantial diffusion barrier to reduce intraepithelial concentrations of capsaicin applied to the lingual surface. Recent studies indicate that <2% of topically applied capsaicin diffuses through the epidermal layer within a 7 min period (Kasting et al., 1997; Magnusson and Koskinen, 2000), allowing us to estimate an intraepithelial capsaicin concentration on the order of 6 μM in the present study. Moreover, anesthetic depression of trigeminal nociceptive activity elicited by lingual capsaicin is another factor explaining the higher threshold for capsaicin to excite Vc neurons (~100 ppm or 330 μM) (Carstens et al., 1998; Dessirier et al., 2000) compared with behavioral capsaicin detection thresholds (0.1–1 ppm or 1 μM) (Simons et al., 2001a, 2002b). Finally, the concentration used presently is comparable with capsaicin concentrations used in relevant human psychophysical studies (198–33,000 μM) of the effect of capsaicin on taste perception (Szolcsanyi, 1977; Lawless and Stevens, 1984; Karrer and Bartoshuk, 1995) not to mention the high concentrations encountered everyday by consumers of spicy food.

Immediately after the end of the 7 min period of capsaicin application, the tastant eliciting the largest response was reapplied, as before, every 3 min for 12 min. This procedure was followed for 34 NTS units in intact rats and for nine additional rats receiving bilateral trigeminal ganglionectomy. As a vehicle control, the effect of identical application of 5% ethanol on tastant-evoked responses of NTS units was determined separately in 12 units. One unit was tested per animal.

Histology. At the conclusion of each experiment, an electrolytic lesion was made at the recording site by passing direct current (6 V) through the microelectrode for 30 sec. Animals were killed by an overdose of pentobarbital delivered through the jugular cannula. The brainstems were removed and postfixed in 10% formalin. At least 2 weeks later they were cut in 50 μm frozen sections and counterstained with neutral red; lesions located within the NTS were identified under the light microscope. The anterior level of the lesion was estimated based on anatomical landmarks (Paxinos and Watson, 1998), and the distance from the midline (in millimeters) was measured. Using these two coordinates, the location of each lesion was transferred onto a representative horizontal brainstem section through the NTS (see Fig. 1*D*). No attempts were made presently to correct for interanimal differences in brain size, variations in the relative size and position of the NTS, or tissue shrinkage during histological processing.

Data analysis. For each neuron, the effect of capsaicin (or vehicle) treatment was assessed by comparing the averaged precapsaicin (or prevehicle) response with tastant-evoked responses elicited immediately, 3, 6, 9, and 12 min after capsaicin (or vehicle) administration using two-way ANOVA (neuron and time as main effects) followed by *post hoc* least significant difference (LSD) multiple comparison tests. In addition, the percentage of suppression and the degree of specificity (entropy, H) were calculated (Smith and Travers, 1979) for each unit, and a correlational analysis was performed to determine whether the magnitude of suppression varied with breadth of neural tuning. An alternative analysis was also conducted in which neurons were classified as narrowly or broadly tuned according to the method of Pfaffmann et al. (1976). An unpaired t test was used to ascertain whether significant differences in the degree of suppression existed between the two groups. To determine whether the gustatory neurons responded to capsaicin (or vehicle) per se, the total number of spikes elicited during each 1 sec or 1 min period before and after capsaicin (or vehicle) delivery were compared using ANOVA followed by *post hoc* LSD tests. For each tastant stimulation condition (before capsaicin and after capsaicin), unit responses were averaged in 1 sec bins to construct averaged peristimulus-time histograms (PSTHs). All data are presented as means ± SE, and $p < 0.05$ was taken as significant.

Plasma extravasation

We investigated the possibility that capsaicin-induced plasma extravasation and localized edema within the lingual epithelium may contribute to taste suppression, using Evans blue. Rats ($n = 14$) were anesthetized with sodium pentobarbital (65 mg/kg, i.p.). Evans blue dye (50 mg/kg) was injected intravenously, and either capsaicin (330 μM) or vehicle (5% ethanol) was applied lingually, in a manner identical to that used in the electrophysiological experiments, for a duration of 7 min. Animals were then perfused intracardially with saline. Digital images were made of the

dorsal surface of the tongue and areas of dye concentration, appearing as blue spots, were counted independently by two blinded investigators. Four additional rats underwent the same procedures except that before capsaicin application, they were subjected to unilateral ($n = 2$) or bilateral ($n = 2$) ganglionectomy. Between-group comparisons were made by ANOVA, with $p < 0.05$ taken as significant. Some tongues were also cut in 100 μm frozen sections and viewed under the light microscope.

Results

Electrophysiology (intact animals)

Unit characterization

Of the 34 units responding to the taste mix and subsequently tested with capsaicin, 24% (8 of 34) responded best to the sucrose stimulus and were thus categorized as sucrose-best, 24% (8 of 34) were characterized as citric acid-best, 26% (9 of 34) were categorized as glutamate-best, and 26% (9 of 34) were categorized as salt-best. The majority (27 of 34) of cells responded to multiple taste stimuli. One salt-best cell also responded to quinine and, because of the infrequency of bitter-sensitive neurons in the rostral NTS, quinine rather than NaCl was tested in this unit. The majority (30 of 34) were spontaneously active at rates usually <5 Hz. Most histologically verified recording sites were at the rostral–lateral border of the gustatory NTS (Fig. 1*D*).

Responses to capsaicin

When analyzed in 1 min bins, there was no significant change in the average firing rate during the 7 min after the onset of the capsaicin stimulus ($F_{(8,297)} = 0.30$; $p = 0.964$). However, when analyzed in 1 sec bins, there was a significant ($F_{(539,18360)} = 1.145$; $p = 0.012$) change in activity, most likely reflecting transient changes in firing rate that appear to be of little physiological significance (Fig. 1*A*). The majority of units (25 of 34) showed no change in activity after the initiation of capsaicin, while five (two salt-best, one each sucrose-best, citric acid-best, and glutamate-best) exhibited a relative increase (>100% above spontaneous levels) in firing rate (Fig. 1*B*). The firing rate of these 5 units was significantly higher ($F_{(8,36)} = 3.2$; $p = 0.007$) than precapsaicin levels at 2 min after the onset of capsaicin, although the mean tastant-evoked response (232 spikes per 30 sec) was significantly ($p = 0.004$) larger than the peak capsaicin-evoked response (89 spikes per 30 sec). In comparing the relative responses to tastants versus capsaicin, it should be noted that only single concentrations of stimuli were used. Four units (two glutamate-best, one each NaCl-best and citric acid-best) showed a significant ($F_{(8,24)} = 5.2$; $p < 0.001$) reduction (to <50% of spontaneous levels) after the onset of capsaicin (Fig. 1*C*).

Effect of capsaicin on taste responses

All taste units. Pooling data from all gustatory units, there was a significant ($F_{(5,144)} = 11.6$; $p < 0.001$) reduction (to 61.5%) in the mean tastant-evoked response after lingual capsaicin treatment compared with the precapsaicin response. At 12 min after capsaicin, the mean response, while still depressed ($p < 0.05$), had begun to return to precapsaicin levels. Pooled data for all 34 units are shown in Figure 2*A*, with the *left panel* showing the mean precapsaicin response, the *middle panel* showing the mean response immediately after capsaicin cessation, and the *right panel* showing recovery of the mean response 12 min after the cessation of capsaicin. The breadth of tuning (*H*) across all cells ranged from 0.13 to 0.94 (mean, 0.75 ± 0.03), and there was no apparent correlation between *H* and the magnitude of capsaicin suppression ($r = 0.096$; $p = 0.596$). Similarly, there was no significant ($p = 0.193$) difference in the mean suppression of the narrowly tuned (to 47%) or broadly tuned (to 63%) cells as classified according to Pfaffmann et al. (1976).

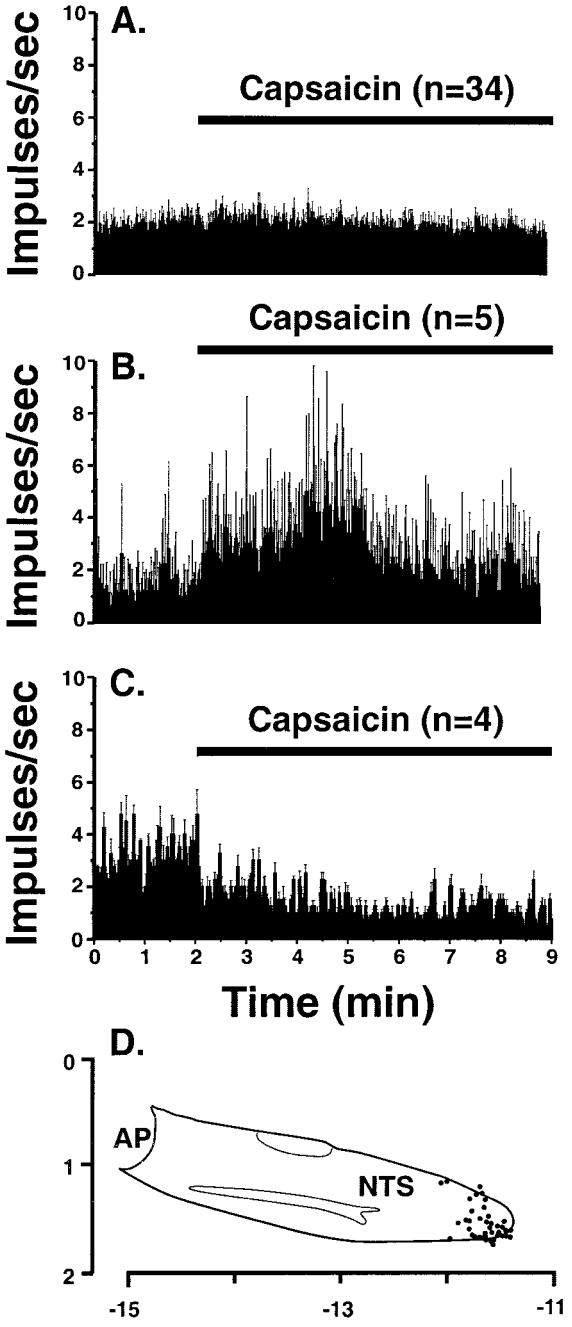

Figure 1. Responses of gustatory NTS units to lingual capsaicin. *A*, Averaged PSTH (bin width, 1 sec) of responses of 34 units to lingual application of capsaicin; stimulus duration is indicated by the *bar above* the PSTH. Error bars indicate SEM. *B*, Averaged PSTH (as in *A*) of responses of 5 units that were excited by capsaicin. *C*, Averaged PSTH (as in *A*) of responses of 4 units that were inhibited by capsaicin. *D*, Recording sites (●) of gustatory NTS units are compiled on a horizontal section through the right NTS, taken from the atlas of Paxinos and Watson (1998). Markers indicate mediolateral (millimeters from midline) and rostrocaudal (from bregma) stereotaxic coordinates. *AP*, Area postrema.

The five NTS units that increased firing rates during application of capsaicin exhibited a similar degree of suppression of tastant-evoked responses immediately after capsaicin (to 55 ± 20% of control). Similarly, the four units that decreased firing rates during capsaicin application exhibited comparable suppression of tastant-evoked responses (to 46 ± 18% of control).

Sucrose-best units. After capsaicin, the mean response of eight sucrose-best units was significantly ($F_{(5,29)} = 4.1$; $p = 0.007$)

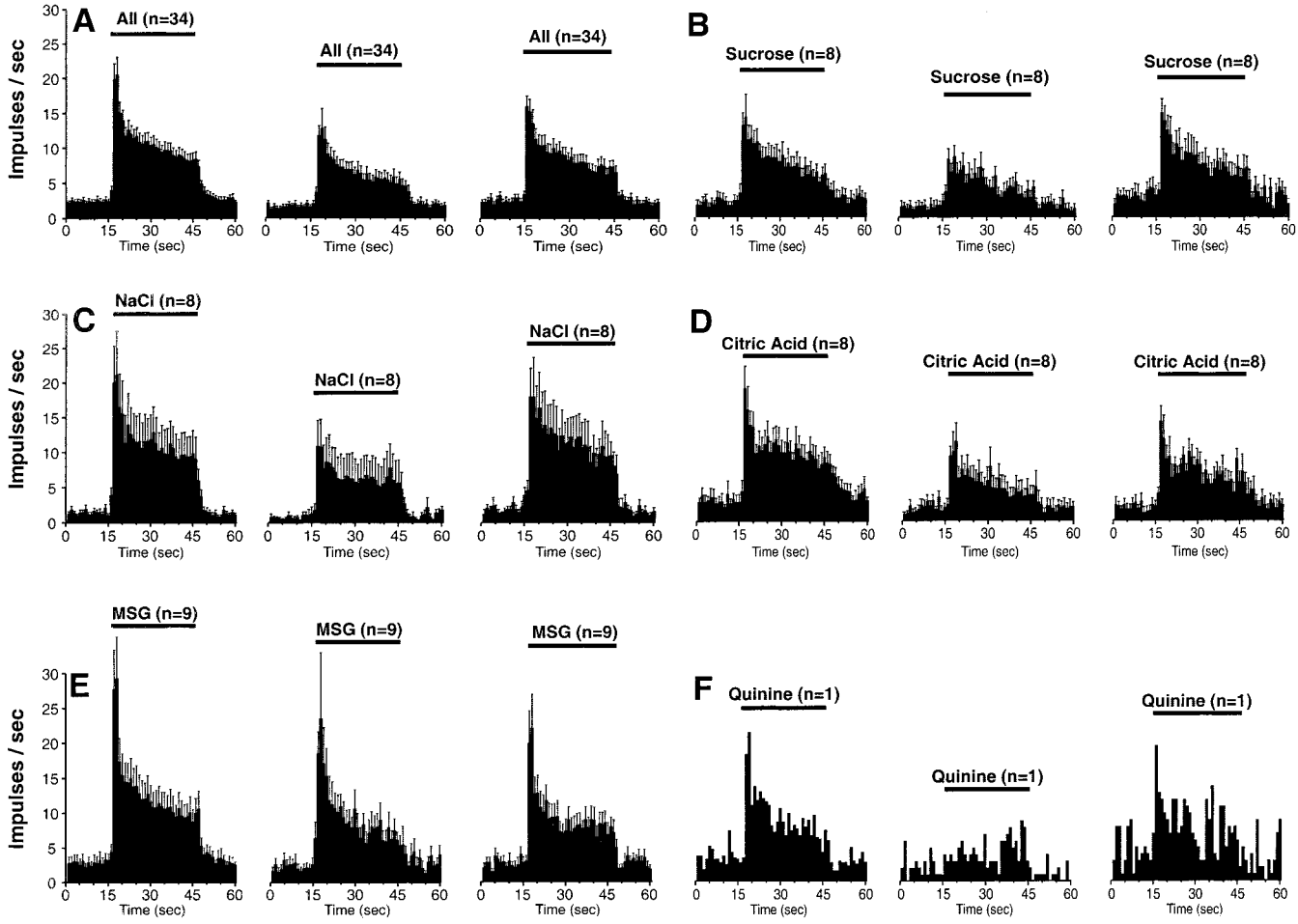

Figure 2. Suppression of tastant-evoked responses of NTS units by oral capsaicin. Each triad of *panels* shows averaged PSTHs of NTS unit responses to the indicated tastant, before capsaicin (left PSTHs), immediately after cessation of capsaicin (middle PSTHs), and 12 min after cessation of capsaicin (right PSTHs). *Horizontal bars* indicate 30 sec duration of tastant stimulus. Error bars indicate SEM. *A*, Data from all 34 units were pooled to show averaged responses to the best tastant. Note suppression of response (*middle panel*; to 57%) immediately after capsaicin with recovery (*right panel*). *B*, Sucrose. Note suppression (*middle panel*; to 63%) immediately after capsaicin with recovery (*right panel*). *C*, NaCl. Note suppression (*middle panel*; to 44%) immediately after capsaicin with recovery (*right panel*). *D*, Citric acid. Note suppression (*middle panel*; to 50%) immediately after capsaicin with partial recovery (*right panel*). *E*, MSG. Note suppression (*middle panel*; to 73%) immediately after capsaicin with little or no recovery (*right panel*). *F*, Quinine; individual example showing that the response of the unit to quinine (*left panel*) was depressed immediately after capsaicin (*middle panel*) with recovery (*right panel*).

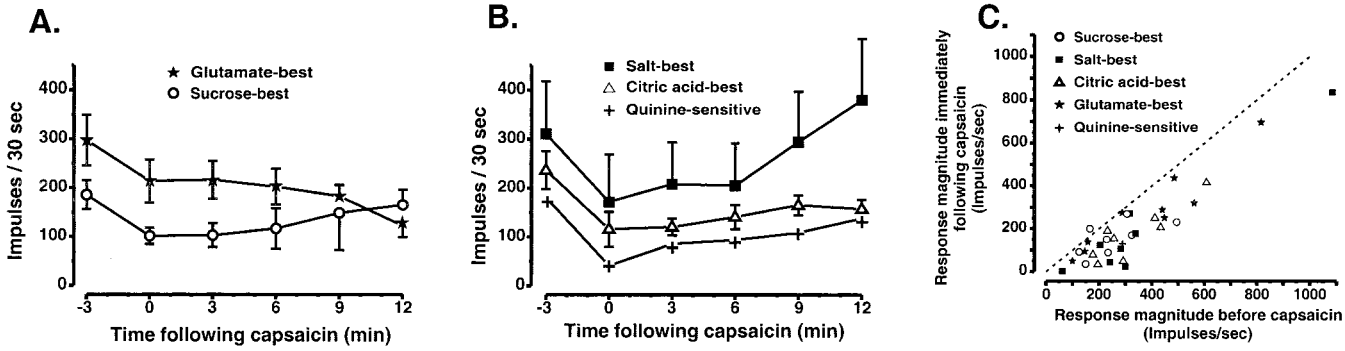

Figure 3. Time course and variability of capsaicin suppression of NTS unit responses to tastants. *A*, Graph plots averaged response of NTS units to glutamate (★) or sucrose (○) versus time relative to capsaicin application. Error bars indicate SEM. *B*, Graph as in *A* for averaged NTS unit responses to NaCl (■) or citric acid (△). Also shown is the response of one unit to quinine (+). *C*, Graph plots the response of each NTS unit to its best tastant before capsaicin (*x*-axis) versus its response to the same tastant immediately after cessation of capsaicin (*y*-axis). *Symbols* indicate the tastant that was tested for each plotted unit. The *dashed diagonal line* indicates no effect of capsaicin on tastant-evoked response.

reduced (to 62.8% of control). Figure 2*B* shows averaged PSTHs of these units before capsaicin, immediately after cessation of capsaicin, and 12 min after capsaicin cessation, respectively. Averaged responses are plotted in Figure 3*A* (○), showing a reduction immediately after capsaicin that began to recover within 6 min and was no longer significantly different from precapsaicin levels at 9 min after capsaicin. Figure 3*C* plots the response of each gustatory unit to its best tastant before capsaicin against its

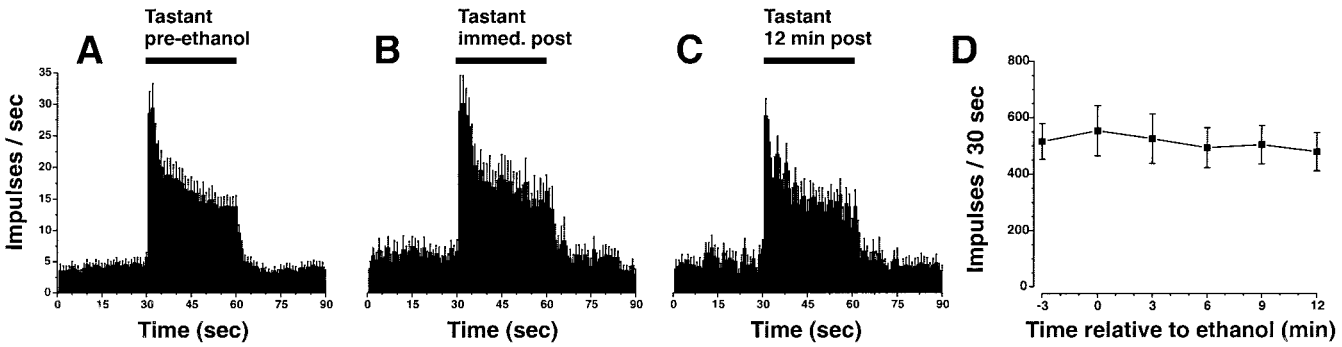

Figure 4. Vehicle controls. *A–C*, Averaged PSTHs of NTS unit tastant-evoked responses ($n = 12$) recorded before (*A*), immediately after (*B*), and 12 min after (*C*) lingual application of vehicle (5% ethanol; format as in Fig. 3). *D*, Graph plots mean tastant-evoked responses of NTS units versus time relative to lingual application of ethanol, to show absence of effect.

response to the same tastant immediately after cessation of capsaicin stimulation. It can be seen that the magnitude of the effect of capsaicin on sucrose-evoked responses varied (range, 15.9–121.2% of control); responses of six of eight units were reduced to ≤75% of control levels.

Salt-best units. The mean response of eight salt-best units to NaCl was significantly ($F_{(5,30)} = 5.1$; $p = 0.002$) attenuated (to 43.6%) immediately after capsaicin (Fig. 2*C*), although there was some variability in the magnitude of suppression (range, 4.8–85.7% of control) (Fig. 3*C*, ■). Within 3 min after the cessation of capsaicin, the response to NaCl began to recover (Fig. 3*B*, ■) and reached precapsaicin levels at 9 min after capsaicin (Figs. 2*C*, *right panel*, 3*B*).

Citric acid-best units. Responses of all eight citric acid-best units were depressed to variable degrees (range, 16.8–82.1% of control) after capsaicin (Fig. 3*C*, △). The mean response of these units to citric acid was significantly ($F_{(5,29)} = 6.2$; $p = 0.001$) reduced (to 49.7%) immediately after capsaicin application compared with the precapsaicin response (Fig. 2*D*), and showed a partial recovery over time after capsaicin (Fig. 3*B*, △). However, the mean response 12 min after capsaicin was still significantly different from the precapsaicin response (Figs. 2*D*, *right panel*, 3*B*).

Glutamate-best units. The mean response of nine units to MSG was significantly ($F_{(4,32)} = 3.8$; $p = 0.013$) reduced (to 72.6%) after lingual capsaicin (Fig. 2*E*, *middle panel*) compared with the precapsaicin level (Fig. 2*E*, *left panel*). The magnitude of effect of capsaicin varied (range, 50.2–95.6% of control) (Fig. 3*C*, ★). However, unlike other tastants, the MSG-evoked responses showed very little recovery after capsaicin (Fig. 3*A*, ★). Indeed, 12 min after the cessation of capsaicin, the response to MSG was not significantly different from the response seen immediately after capsaicin administration (Figs. 2*E*, *right panel*, 3*A*).

Quinine-responsive unit. One NaCl-best unit was tested with quinine, which also evoked a response (Fig. 2*F*, *left panel*) that was substantially suppressed (to 45.8%) immediately after capsaicin pretreatment (Figs. 2*F*, *middle panel*, 3*C*, +) with subsequent recovery (Figs. 2*F*, *right panel*, 3*B*, +).

Vehicle controls. Twelve units (five citric acid-best, three sucrose-best, three salt-best, and one glutamate-best) were tested with vehicle (5% ethanol). There was no significant change in the mean firing rate of these units during application of vehicle ($F_{(8,99)} = 0.172$; $p = 0.994$) nor were tastant-evoked responses significantly affected ($F_{(5,55)} = 1.660$; $p = 0.160$) after vehicle (104.6% of mean prevehicle response). Figure 4*A–C* shows averaged PSTHs of tastant-evoked responses of the NTS units before, immediately after, and 12 min after ethanol, and Figure 4*D* shows the absence of an appreciable change in response relative to the time of ethanol application.

Electrophysiology (ganglionectomized animals)

Recordings were made from nine gustatory NTS units (two sucrose-best, three NaCl-best, three citric acid-best, and one glutamate-best) in nine rats that had received bilateral trigeminal ganglionectomy. All units were histologically localized to the rostral–lateral border of the NTS (Fig. 5*F*). Figure 5*A–C* shows averaged PSTHs of responses before capsaicin (Fig. 5*A*), immediately after capsaicin (Fig. 5*B*), and 12 min after capsaicin (Fig. 5*C*). After lingual capsaicin administration, the mean tastant-evoked response of these units was significantly suppressed ($F_{(5,39)} = 12.81$; $p < 0.001$) to a level (65.4%) comparable with that seen in intact rats (compare Figs. 2*A*, *middle panel*, and 5*B*). As in the intact animals, there was some variability in the time course of recovery from capsaicin suppression (Fig. 5*D*) as well as the degree of suppression of the best tastant-evoked response of each unit (Fig. 5*E*).

Plasma extravasation

Each of the nine intact rats receiving lingual capsaicin exhibited plasma extravasation manifested by a punctate distribution of dye spots in a pattern consistent with that of fungiform papillae (Miller and Preslar, 1975) (Fig. 6*A*). In transverse frozen sections through capsaicin-treated tongues, areas of dye accumulation were microscopically verified to be fungiform papillae, with little or no dye seen in surrounding tissues (Fig. 6*C*). This is in marked contrast to the five animals receiving vehicle, in which none of the tongues displayed signs of inflammation or extravasation (Fig. 6*B*). In the four animals subjected to trigeminal ganglionectomy, the pattern of dye distribution was indistinguishable from that seen in the intact rats. Indeed, the mean number of stained papillae in intact rats (110 ± 5) and ganglionectomized rats (101 ± 6) was not significantly different (LSD; $p = 0.211$), whereas both groups were found to have significantly ($F_{(2,15)} = 135.6$; $p < 0.001$) higher counts compared with ethanol-treated controls (0 ± 0).

Discussion

The main finding of this study is that capsaicin significantly depressed responses of gustatory NTS units to each of the five tastants tested, thus demonstrating a physiological interaction between oral irritation and taste that is presumably expressed at the perceptual level. The depressant effect of capsaicin was not signifi-

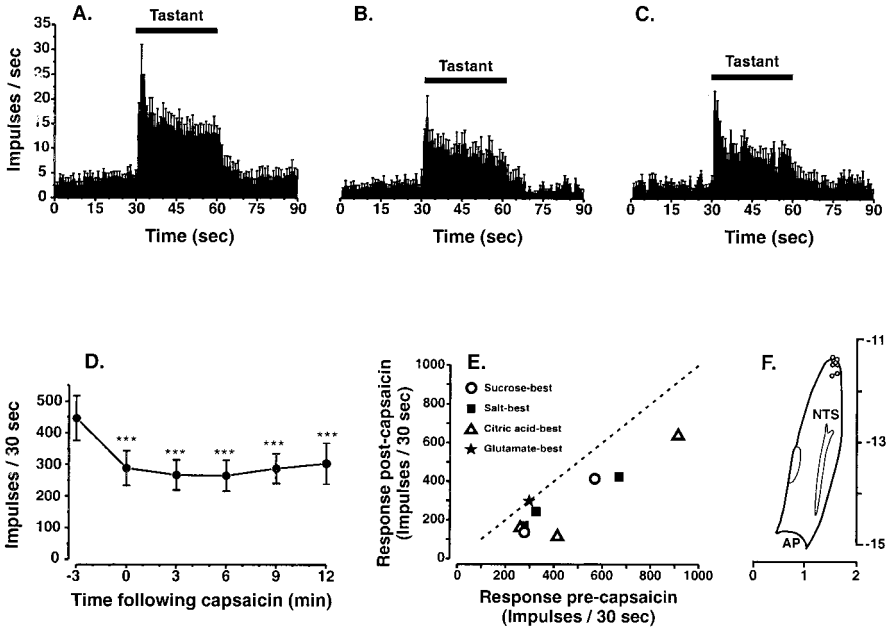

Figure 5. Suppression of NTS unit responses to tastant stimuli in animals with bilateral ganglionectomy. *A–C*, Data from nine neurons pooled to show averaged responses to the best tastant before capsaicin (*A*), immediately after capsaicin (*B*), and 12 min (*C*) after the cessation of capsaicin. *Horizontal bars* indicate 30 sec duration of tastant stimulus. Error bars indicate SEM. *D*, Graph plots averaged tastant-evoked response of NTS units versus time relative to capsaicin application. Error bars indicate SEM. $^{***}p < 0.001$. *E*, Graph plots the response of each NTS unit to its best tastant before capsaicin (*x*-axis) versus its response to the same tastant immediately after cessation of capsaicin (*y*-axis). *Symbols* indicate the tastant that was tested for each plotted unit. The *dashed diagonal line* indicates no effect of capsaicin on tastant-evoked response. *F*, Recording sites (○) of gustatory NTS units compiled onto a horizontal section through the right NTS. Graph as in Figure 1*D*.

cantly different in intact compared with trigeminal ganglionectomized animals, arguing against a trigeminally mediated central effect. It is more likely that capsaicin influences taste processing at a peripheral site, possibly via mechanisms related to the punctate distribution of plasma extravasation in the tongue that was observed after capsaicin. A secondary finding was that a fraction of the gustatory NTS units responded during capsaicin application alone, although these responses were smaller in magnitude than those elicited by the best tastant, suggesting either that such units signal oral irritation or that capsaicin has gustatory properties.

Sites and mechanisms of capsaicin suppression of taste

Consistent with many previous studies of central gustatory neurons, NTS units were categorized according to the tastant that elicited the relatively largest (best) response, although they typically responded to two or more of the five tastants. Responses elicited by the best tastant of each unit were in nearly all cases markedly suppressed immediately after capsaicin application, with some variability in the magnitude and duration of the effect that was independent of the breadth of tuning for a given neuron. This is in contrast to a previous study showing capsaicin to have differential effects on NaCl responsiveness in narrowly versus broadly tuned chorda tympani fibers (Osada et al., 1997). This difference might reflect the high degree of convergence of primary afferents within the NTS in which the suppressive effect of capsaicin on chorda tympani fibers dominates.

Overall, tastant-evoked responses were reduced to 61.5%, a level that was not significantly different from that of capsaicin suppression in rats with bilateral trigeminal ganglionectomy (65.4%). These results indicate that a centrally mediated mechanism dependent on capsaicin activation of trigeminal afferents is not critical to the depressant effect of capsaicin on tastant-evoked NTS unit responses. The gustatory NTS is densely innervated by opioid peptide- and GABA-containing neurons (Lynch et al., 1985; Davis, 1993; Davis and Kream, 1993), and GABA exerts inhibitory effects on gustatory NTS neurons (Bradley and Grabauskas, 1998). We therefore cannot rule out the possibility that capsaicin may excite extratrigeminal pathways to induce a central suppression of taste processing via release of these inhibitory neurotransmitters.

A peripheral action of capsaicin on taste transduction is more parsimonious with the present results. Previous studies have shown that electrical stimulation of the lingual nerve (Wang et al., 1995) or chemical excitation of the tongue by capsaicin (Osada et al., 1997) resulted in decreased responsiveness of NaCl-sensitive primary afferent fibers in the chorda tympani, a finding that is consistent with the present results showing capsaicin suppression of NaCl-evoked NTS unit responses. In this regard, it was reported recently that capsaicin inhibits the activation of voltage-gated ion channels in trigeminal ganglion neurons (Liu et al., 2001) and in taste receptor cells responsive to NaCl (S. A. Simon, personal communication), suggesting that capsaicin may inhibit the generation of action potentials in these cells as well as primary gustatory afferents. Whether the responses of chorda tympani fibers or taste receptor cells to other tastants are also inhibited by capsaicin, as observed presently for gustatory NTS neurons, remains to be determined.

In addition to possible direct actions of capsaicin on taste receptor cells, capsaicin evokes an axon reflex resulting in the release of substance P and other neuroactive peptides from the peripheral terminals of nociceptors (Cao et al., 1998) causing localized plasma extravasation and edema (Holzer, 1998). We observed that capsaicin elicited a punctate pattern of plasma extravasation in the tongue (Fig. 6*A*,*C*) consistent with the distribution of fungiform papillae (Miller and Preslar, 1975). This finding was consistent in both intact and ganglionectomized rats and suggests a mechanism whereby localized plasma extravasation and edema in taste papillae could result in the closure of taste pores and thereby impede access of tastant molecules to taste receptor cells. An additional possibility involves an irritant-induced contractile mechanism in mouse taste buds that has been hypothesized to protect against potentially damaging effects of noxious stimuli (Mattern and Paran, 1974). Finally, there is a relatively high density in and around taste buds of substance P-containing fibers (Nagy et al., 1982; Nishimoto et al., 1982; Finger, 1986) that are primarily of trigeminal origin (Nagy et al., 1982). Moreover, the neurokinin-1 receptor, to which substance P preferentially binds, is expressed in the basolateral membrane of taste receptor cells but not in the surrounding epithelium or in primary gustatory neurons (Chang et al., 1996). It is therefore possible that substance P, released via the axon reflex (Holzer, 1998), could act directly on taste receptor cells to alter their gustatory responsiveness.

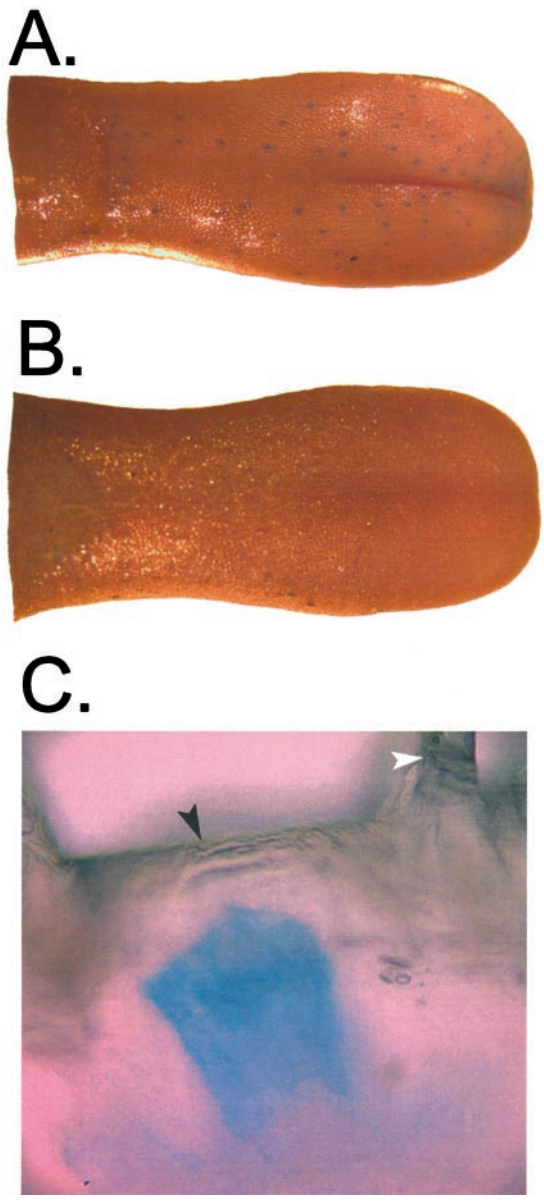

Figure 6. Capsaicin-induced plasma extravasation. *A*, Photograph of rat tongue treated previously with topical capsaicin. Note accumulation of blue dye within taste papillae across the lingual surface. *B*, Photograph of rat tongue pretreated with ethanol. *C*, Photomicrograph of section through fungiform papillae (100× magnification). The *black arrowhead* indicates the dorsal lingual surface, whereas the *white arrowhead* shows a filliform papilla. Note that blue dye accumulates within the taste bud, with little or none in surrounding tissues.

Perceptual correlates

Regular consumers of spicy foods often contend that pungent spices enhance the flavor of foods, whereas infrequent consumers claim that taste is suppressed (Lawless et al., 1985). Psychophysical evidence supports an inhibitory effect of capsaicin on certain suprathreshold taste sensations, particularly sweetness (Lawless et al., 1985; Cowart, 1987; Prescott et al., 1993; Prescott and Stevenson, 1995) and bitterness (Lawless and Stevens, 1984; Cowart, 1987). We recently reported significant reductions in the perceived intensity of sweetness, bitterness, and umami (from MSG) after capsaicin pretreatment; salt and sour qualities were unaffected (Simons et al., 2002a). These psychophysical findings are partly at odds with the present results, which show significant suppression of NTS unit responses to all tastants tested. In particular, the marked suppression by capsaicin of NaCl- and citric acid-evoked NTS unit responses (to 44 and 50% of control, respectively) is inconsistent with the psychophysical observations that capsaicin does not affect the perceived intensity of salt or sour taste qualities. This disparity may be explained by the higher capsaicin concentration used presently compared with that used in human studies (109 μM) or by species differences that may also contribute.

In the present study, the capsaicin vehicle (5% ethanol) had no effect on the firing rates of NTS units or on their responses to tastants. It has been reported previously that the responses of NaCl-sensitive chorda tympani fibers were slightly reduced when the NaCl was applied in mixture with 5% ethanol compared with their responses to NaCl alone (Osada et al., 1997). Any minor effect of ethanol on the sensitivity of chorda tympani fibers to sapid stimuli thus appears to be lost at the level of the NTS and cannot account for the significant depressant effect of capsaicin on tastant-evoked responses.

NTS unit responses to capsaicin

Although most gustatory NTS units were unaffected by capsaicin, a fraction exhibited small but significant excitatory or inhibitory responses (Fig. 2), possibly via convergent input from capsaicin-sensitive trigeminal fibers. Gustatory NTS neurons are known to be excited by innocuous somatosensory stimuli (Ogawa et al., 1984, 1988; Hayama et al., 1985; Travers and Norgren, 1995), and anatomical studies have shown projections to the NTS from trigeminal afferents (Jacquin et al., 1982; Hamilton and Norgren 1984; Marfurt and Rajchert, 1991) as well as from neurons in the Vc and paratrigeminal nucleus (Menétrey and Basbaum, 1987; Saxon and Hopkins, 1998). A second possibility is that some gustatory NTS units receive input directly from capsaicin-sensitive chorda tympani fibers (Okuni, 1977), although other studies indicate that chorda tympani fibers are not capsaicin sensitive (Silver et al., 1985; Hiura et al., 1990). A third possibility is that the capsaicin-evoked NTS unit responses reflect a gustatory component of capsaicin, which is sometimes reported to have a bitter taste, particularly at the back of the tongue (Lawless and Stevens, 1988; B.G. Green, personal communication).

In summary, the present finding that capsaicin significantly attenuates responses of gustatory NTS neurons to tastants provides a solid physiological basis to explain the suppression of certain tastes by spices in food.

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