Activation of Neurons in Rat Trigeminal Subnucleus Caudalis by Different Irritant Chemicals Applied to Oral or Ocular Mucosa

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Carstens, E., Nicole Kuenzler, and H. O. Handwerker. Activation of neurons in rat trigeminal subnucleus caudalis by different irritant chemicals applied to oral or ocular mucosa. J. Neurophysiol. 80: 465–492, 1998. To investigate the role of trigeminal subnucleus caudalis in neural mechanisms of irritation, we recorded single-unit responses to application of a variety of irritant chemicals to the tongue or ocular mucosa in thiopental-anesthetized rats. Recordings were made from wide dynamic range (WDR) and nociceptive-specific units in superficial layers of the dorsomedial caudalis (0–3 mm caudal to obex) responsive to mechanical stimulation and noxious heating of the ipsilateral tongue (“tongue” units) and from WDR units in ventrolateral caudalis (0–2 caudal to obex) responsive to mechanical and noxious thermal stimulation of cornea-conjunctiva and frequently also surrounding skin (“cornea-conjunctival” units). The following chemicals were delivered topically (0.1 ml) onto the dorsal anterior tongue or instilled into the ipsilateral eye: capsaicin (0.001–1% = 3.3 × 10⁻² to 3.3 × 10⁻⁴ M), ethanol (15–80%), histamine (0.01–10% = 9 × 10⁻¹ to 9 × 10⁻³ M), mustard oil (allyl-isothiocyanate, 4–100% = 4 × 10⁻¹ to 10 M), NaCl (0.5–5 M), nicotine (0.01–10% = 6 × 10⁻¹ to 6 × 10⁻⁴ M), acidified phosphate buffer (pH 1–6), piperine (0.01–1% = 3.5 × 10⁻³ to 3.5 × 10⁻⁴ M), serotonin (5-HT; 0.3–3% = 1.4 × 10⁻¹ to 1.4 × 10⁻² M), and carbonated water. The dose-response relationship and possible tachyphylaxis were tested for each chemical. Of 32 tongue units, 31 responded to one or more, and frequently all, chemicals tested. The population responded to 75.3% of the various chemicals tested (±10 per unit). The incidence of responses was independent of the order of chemicals tested, except for capsaicin, which reduced subsequent responses. Responses to histamine, nicotine, 5-HT, and ethanol had a more rapid onset and shorter duration compared with capsaicin, acid, and mustard oil. Responses to all chemicals increased in a dose-related manner. Successive responses to repeated application decreased significantly for nicotine, 5-HT, capsaicin, and piperine. Spontaneous firing increased significantly 5–10 min after initial application of capsaicin. Of 31 corneal-conjunctival units, 29 responded to one or more chemicals, and the population responded to 65% of all chemicals tested. Responses increased in a dose-related manner for all chemicals, and successive responses decreased significantly for histamine, nicotine, ethanol, acid, and capsaicin. Responses of tongue units to histamine and nicotine were reduced significantly by cetrizine (H₁ antagonist) and mecamylamine, respectively. Mecamylamine also significantly reduced responses of corneal-conjunctival units to nicotine. Different classes of irritant chemicals contacting the oral or ocular mucosa can activate individual sensory neurons in caudalis, presumably via independent peripheral transduction mechanisms. Multireceptive units with input from the tongue or cornea-conjunctiva exhibited a similar spectrum of excitability to different irritant chemicals. Such neurons would not be capable of discriminating among different chemically evoked irritant sensations but could contribute to a common chemical sense.

INTRODUCTION

Despite the widespread consumption of food spices and tobacco products that contain chemicals that irritate oral and ocular mucosa, little is known about the central neural mechanisms underlying the resulting irritant sensations. Parker (1912) proposed the concept of a “common chemical sense” elicited by noxious chemicals. This was mediated by free nerve endings and served a protective function. A variety of chemicals elicit irritation or pain when delivered to the oral or ocular mucosa (reviewed in Green and Lawless 1991; Green et al. 1990) (see DISCUSSION). Sensations from some of these irritants (capsaicin, NaCl, citric acid, cinnamon aldehyde, and menthol) are cross-desensitized by capsaicin (Cliff and Green 1996; Dessirier et al. 1997; Green 1991), suggesting that the irritation is mediated partly by a common population of capsaicin-sensitive fibers. It is currently uncertain whether different qualities of oral or ocular irritation can be discriminated (see DISCUSSION) or to what extent central trigeminal neurons can be activated by one or more irritant chemical. The present study was undertaken to address this latter question.

Irritant chemicals in the oral cavity presumably activate chemosensitive nociceptors the free endings of which are located in the mucosal epithelium and lamina propria (Hol and Lundy 1984). Irritants contacting the ocular surface activate epithelial free nerve endings of nociceptors having thinly myelinated or unmyelinated fibers (Maclver and Tanelian 1993a,b). Single-fiber recordings from lingual (Bryant and Moore 1995; Hellekant 1965; Komai and Bryant 1993; Lundy and Contreras 1994; Okuni 1978; Sostman and Simon 1991; Wang et al. 1993) or ciliary nerves (Belmonte and Giraldez 1981; Belmonte et al. 1991; Chen et al. 1995, 1997; Gallar et al. 1993; Maclver and Tanelian 1993b; Tanelian 1991), indicate that polymodal nociceptor and mechanically insensitive afferents can respond to irritant chemicals (acetylcholine, nicotine, NaCl and other salts, acid, capsaicin, and CO₂) although the degree of chemoselectivity of individual fibers is currently uncertain. Recent patch-clamp studies of small-diameter trigeminal ganglion neurons (Liu and Simon 1994, 1996a–c; Liu et al. 1993, 1997) suggest that at least some are capable of responding to more than one class of irritant chemical.

Afferent fibers innervating the tongue pass via the lingual
nerve of the mandibular division, whereasafferent fibers from the cornea-conjunctiva pass via the ciliary nerve in the ophthalmic division, to terminate in brain stem trigeminal
subnucleus caudalis, interpolaris, oralis, and principalis in a somatotopically organized manner with the head inverted and
may contribute to irritant sensations (Norgren 1984). Electrophysiological studies indicate that a substantial fraction
of neurons in subnucleus caudalis responds differentially or exclusively to noxious stimulation of intraoral tissue, cornea
or face (e.g., Amano et al. 1986; Bushnell et al. 1984; Chiang et al. 1994; Hu 1990; Hu et al. 1981; McHaffie et al. 1994;
Price et al. 1976; Raboisson et al. 1995; Renehan et al. 1986; Sessle et al. 1981, 1986; Yokota 1975; Yokota and
also respond to noxious stimuli, and it was suggested that these may represent a rostral extension of lamina I of sub-
nucleus caudalis (Hayashi and Tabata 1989b; Schults 1992b). Furthermore, neurons in more rostral trigeminal
subnuclei interpolaris (Hayashi et al. 1984; Ohya 1992) and oralis (Dallel et al. 1990, 1996; Hayashi and Tabata 1989a;
Hu and Sessle 1984; Jacquin and Rhoades 1990; Raboisson et al. 1991; Sessle and Greenwood 1976) also respond to
noxious orofacial stimuli although the incidence of such noc-
responsive neurons is lower compared with caudalis. A pri-
mary role for subnucleus caudalis in signaling pain is sup-
ported by recent immunohistochemical data showing that
neurons expressing c-fos after noxious orofacial stimulation
are located in somatotopically appropriate regions of sub-
nucleus caudalis but not more rostral trigeminal subnuclei
(Anton et al. 1992b; Bereiter et al. 1994; Carstens et al.
1995; Coimbra and Coimbra 1994; Lu et al. 1993; Mineta et al. 1995; Meng and Bereiter 1996; Strassman and Vos
1993).

To date there have been relatively few studies of the re-
sponses of trigeminal neurons to irritant chemicals in general (Amano et al. 1986; Ebersberger et al. 1997; Hu et al. 1992;
Meng et al. 1997; Mosso and Kruger 1973; Peppel and Anton 1993; Raboisson et al. 1991, 1995; Yu et al. 1993) and,
to our knowledge, none concerning neuronal responses to
application of irritant chemicals onto the surface of the
tongue. We recently reported that application of different irritant chemicals (nicotine, capsaicin, pipeline, and hist-
aamine) onto the dorsal tongue resulted in a similar distribution of c-fos-immunoreactivity in the superficial layers of the
dorsomedial aspect of trigeminal subnucleus caudalis, in ad-
dition to other brain stem areas (Carstens et al. 1995). Others
have reported that noxious mechanical or chemical stimulation of the cornea produces two distributions of c-fos-immu-
noreactivity, one in the ventrolateral aspect at the transition
of rostral caudalis to interpolaris, and a more caudal distribu-
tion in the ventrolateral dorsal horn of the upper cervical
spinal cord (Bereiter and Bereiter 1996; Bereiter et al. 1994;
Lu et al. 1993; Martinez and Belmonte 1996; Meng and
Bereiter 1996; Strassman and Vos 1993). A limitation of the
c-fos method is that it cannot distinguish whether one
and the same neuron is activated by different chemicals or
if there are separate populations of “chemospecific” neurons
grouped near one another with each responding only to
one chemical. For this reason, electrophysiological experiments
were undertaken to determine if single neurons in superficial
dorsomedial caudalis can respond to application onto the
tongue of a variety of irritant chemicals that are presumed
to act via different peripheral transduction mechanisms
(Barnd and Bryant 1994; M. Kress and P. W. Reeh, unpub-
lished data). Our aim was to sample a variety of irritant
chemicals, some of which act at specific molecular receptors
(e.g., histamine, capsaicin, serotonin, and nicotine), whereas
others have nonspecific or unknown effects on the nociceptor
terminal membrane. For comparison, we similarly tested if
neurons in the ventrolateral aspect of caudalis receiving input
from the cornea-conjunctiva respond to different irritant
chemicals applied to the ocular surface. Abstracts of this
work have appeared elsewhere (Carstens et al. 1996; Kuen-
zler et al. 1996).

METHODS

Surgery

Experiments were conducted using 30 adult male Wistar rats
(300–450 g). Anesthesia was induced with thiopental (120 mg/
kg ip). Supplemental doses of thiopental (20–60 mg/kg ip) were
administered as necessary to maintain a constant level of anesthesia
as assessed by areflexia, absence of any organized movements
(e.g., of the tongue), and absence of heart rate changes (as
monitored by electrocardiogram) on noxious stimulation. A tracheotomy
was performed, and a catheter was placed in the jugular vein for
infusion of isotonic saline and paralytic agent. Scopolamine
(50 mg/kg sc) was given. Core temperature was monitored and maintained
at ~37°C by a feedback-controlled infrared lamp. During
recording, the animals were paralyzed (Pancuronium, bolus injection
of ~0.5 mg/0.25 ml iv) and mechanically ventilated at a rate and
tidal volume sufficient to maintain end-tidal CO2 (monitored with a Datex infrared CO2 analyzer) at 4–5%. The anesthetic level
was checked periodically when the effect of the paralytic agent
waned.

The upper cervical spine and occipital bone were exposed by
midline incision, and the base of the cerebellum, lower brain stem,
and C1 spinal cord exposed by removal of the atlas and caudal-
most part of the occipital bone. The animal was placed in a stereo-
taxic frame, and the head fixed in a ventroflexed position. The
upper cervical spine was rigidly held in place with a vertebral clamp. The exposed surface of the brain stem was covered with
agar. A small opening was made in the hardened agar to expose
the brain stem, which then was bathed in warmed isotonic saline.

Single-unit recording

The recording microelectrode was advanced into the brain stem in 5-μm steps using a piezoelectric microdrive. In most
experiments, a Teflon-insulated tungsten microelectrode (WPI, ~10
MΩ) was used, while in a few experiments a glass-coated carbon-
fiber microelectrode was used. Extracellular single-unit activity

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was amplified and displayed by conventional means and fed via a Microstar analog-digital converter to a computer. Unitary action potentials were discriminated, and instantaneous frequency sampled continually, using software developed in Erlangen (Forster and Handwerker 1990).

Recordings were made from separate unit populations: units in superficial layers of the dorsomedial trigeminal subnucleus caudalis that responded to pressure and pinch stimuli applied to the tip or side of the ipsilateral tongue (‘‘tongue’’ units) and units in ventrolateral caudalis that responded to tactile stimulation of the cornea-conjunctiva (‘‘corneal-conjunctival’’ units). In 11 rats, only units with tongue input were studied, whereas in 17 rats, a unit with input from tongue as well as another unit with input from the cornea-conjunctiva were studied. In two rats, only units with corneal input were studied. We believe it is unlikely that the prior recording from a tongue unit influenced the responses of the subsequently recorded corneal units (or vice versa) because units with convergent afferent input from the tongue and cornea were never observed and because we detected no obvious differences in data obtained from the experiments in which cornea units were recorded first compared with those in which tongue units were recorded first. In about one-half (10/19) of experiments with corneal unit recordings, separate units with input from the left and right cornea were recorded. We also did not detect any marked differences in data obtained from the first compared with second corneal unit recorded in the same experiment. Before recording corneal-conjunctival units on one side, the ocular surface was covered with an ointment (Bepanthen, Roche) to prevent desiccation.

The search for units with input from the tongue was restricted to the area ~0–2 mm caudal to the obex and ~1.5 mm lateral to the midline, based on earlier c-fos immunohistochemical mapping studies (Carstens et al. 1995; Strassman and Vos 1993). Units responsive to mechanical stimulation of the tongue were identified readily at depths ranging from 50 to 300 μm, and no deeper than 600 μm, below the surface of the brain stem. To search for units responsive to mechanical stimulation of cornea-conjunctiva, microelectrode penetrations were made 1–3 mm caudal from obex and 2.5 mm lateral to the midline and at depths ranging from 1–2.5 mm below the medullary surface.

**Characterization of tongue units**

The mouth was held open to access the dorsal anterior tongue, which was frequently moistened with isotonic saline to prevent desiccation. Only units that responded to mechanical (pressure, pinch) stimulation of the tongue, and additionally to noxious thermal stimulation (topical application of hot water at 48–54°C water to the dorsal tongue), were selected for further study. For units with no spontaneous activity, any stimulus-evoked discharge was considered to be a response. For units with spontaneous activity, a response was generally considered as a two- to threefold increase in firing rate during stimulus presentation; this subjective definition was borne out by subsequent statistical analysis (see further text). Units’ mechanosensitive receptive fields were mapped approximately using pressure-pinch stimulation with forceps or small arterial clamps exerting different forces and with von Frey hairs. The extent of lingual mechanical receptive fields was difficult to map precisely because the tongue was not stabilized. All units were additionally tested for sensitivity to cooling the tongue with a cotton ball cooled to ~4°C with an inert cold spray used routinely in dentistry (Kaltespray, Schein-Dentina); an uncooled cotton ball served as a mechanical control stimulus. Units were classified as nociceptive-specific if they responded only to noxious levels of pressure-pinch stimuli (as judged by application of the same stimulus to the experimenter’s tongue) and did not respond to the cold stimulus. These were classified as wide dynamic range (WDR) type if they responded to the cold stimulus and/or to nonnoxious mechanical pressure. Usually only one unit with tongue input was studied per animal; in four cases a second unit on the opposite side also was studied.

**Characterization of corneal-conjunctival units**

Mechanical brush, tap, and blunt pressure stimuli delivered to the cornea-conjunctiva and periorbital tissue were used to search for units. When a responsive unit was isolated, its mechanical receptive field was mapped more completely with graded von Frey hairs, and responsiveness to cooling was tested by applying a cold cotton ball to the eye. Only units the mechanical receptive field of which included the cornea and that additionally responded to noxious thermal stimulation of the cornea-conjunctiva by topical application of hot water (48–54°C) onto the surface of the eye (i.e., WDR type), were selected for further study.

**Chemical stimulation of the tongue**

Chemicals were applied topically by syringe onto the dorsal surface of the anterior tongue in a standard volume of 0.1 ml. The fluid volume covered an area of ~1 mm in diameter on the tip of the tongue bilaterally. In early experiments, a strip of Parafilm was placed underneath the tongue to prevent chemicals from reaching underlying tissue. However, because the head was ventroflexed, excess fluid dripping off of the dorsal surface of the tongue did not visibly contact tissue beneath the tongue so that the Parafilm strip was not used in later experiments. Each chemical was left on for 60 s, after which the tongue was rinsed with isotonic saline (0.9%). All chemicals were delivered at room temperature to avoid any confounding effect of cooling in the units that were cold sensitive. In a few cases, we also delivered chemicals continually at a constant flow rate and did not observe any marked prolongation in response duration, although this requires further investigation.

**Chemical stimulation of cornea-conjunctiva**

A volume of 0.1 ml of each noxious chemical was instilled into the eye by syringe. After 60 s, the eye was rinsed in a similar manner with isotonic saline (0.9%). No attempt was made to stimulate the cornea in isolation, so it is assumed that instilled chemicals activated receptors in ocular mucosa of the cornea and/or conjunctiva including the eyelid inner surface. The instilled fluid volume was held in place by surface tension, and no fluid was observed visibly to contact surrounding skin. Isotonic saline per se did not excite units. However, many units that were sensitive to mechanical stimulation of the cornea-conjunctiva or eyelid responded during physical application of the saline or noxious chemical due to direct activation of mechanoreceptors. These mechanically evoked discharges were always brief and restricted to the stimulus period (<2 s) and were in all cases readily distinguishable from chemically evoked responses that occurred later. When present, the mechanically evoked component of the response was subtracted from longer-latency discharges in analyzing unit responses to noxious chemicals.

**Chemicals**

The following chemicals were used routinely: capsaicin (0.001–1% = 3.3×10⁻⁸ to 3.3×10⁻⁴ M; diluted in dH₂O from a stock solution of 1% in 80% ethanol; Sigma, Fluka), ethanol (EtOH; 15–80%, Merck), histamine (0.01–10% = 9×10⁻⁴ to 9×10⁻¹ M in 0.9% NaCl; Sigma), mustard oil (allyl-isothiocyanate, 4–100% = 4×10⁻⁴ to 10 M, direct or diluted in paraffin oil; Merck), NaCl (0.5–5 M in dH₂O), nicotine (0.01–10% = 6×10⁻⁴ to 6×10⁻¹ M in 0.9% NaCl; Sigma), buffer solutions at preset pH values (pH range 1–6; Fisher Scientific), piperine (0.01–1% =
3.5 × 10⁻² to 3.5 × 10⁻⁴ M, diluted in dH₂O from a stock solution of 1% in 80% ethanol (Sigma), and serotonin 5-hydroxytryptamine (5-HT; 0.3 ± 4.9 × 10⁻⁶ to 1.4 × 10⁻⁴ M in 0.9% NaCl; Sigma). In four experiments commercially available carbonated water (Cascada, pH 6.1) was used. Finally, in many experiments the H1 receptor antagonist cetirizine (Zyrtec, 0.1–1%; direct or diluted in 0.9% NaCl; UCB Chemie) (Simons and Simons 1991) or the nicotinic antagonist mecamylamine (0.1% = 4.9 × 10⁻⁵ M, in 0.9% NaCl; Sigma) also were used. The pH of all solutions except acidified buffer and carbonated water was neutral.

Sequential chemical stimulation

To determine if individual units respond to different chemicals delivered sequentially, it was imperative to determine if a given chemical induced long-lasting changes in the excitability of chemosensitive receptors in the tongue or ocular surface. Pilot experiments revealed that sufficiently high concentrations of capsaicin, piperine and mustard oil often desensitized the tongue, such that the unit no longer responded to subsequent application of any chemical. None of the other chemicals tested produced a marked desensitization. Therefore the different chemicals were delivered in a pseudorandom sequence except that capsaicin, piperine, and/or mustard oil were tested last. However, because later experiments focused on the effect of the H1 antagonist applied to the tongue, histamine was the first chemical tested in the majority (69%) of units with afferent input from the tongue.

Experimental design and data analysis

For each chemical tested, we delivered different concentrations to establish a dose-response relationship and/or a constant concentration repeatedly, to check for tachyphylaxis or sensitization. Chemicals were delivered at a 5-min interstimulus interval, which was chosen as a compromise to test as many of the 10 chemicals as possible for each unit. Generally, each chemical was delivered in ascending order of concentration so that the dose-response relationship was determined first. If the unit responded robustly at a given concentration, the chemical was then delivered successively at that concentration two to four times to check for tachyphylaxis.

After collecting data for one chemical, the next chemical was similarly tested. In some cases, tachyphylaxis was tested first using a suprathreshold concentration and the dose-response relationship was either determined later or not at all. Thus we could not obtain both dose-response and tachyphylaxis data for all units. For a given unit, we attempted to test as many of the 10 chemicals as possible in this manner. Data for each chemical were pooled to generate profiles of the time course of mean responses (Figs. 4 and 5), population dose-response relationships, and response levels over repeated trials (Figs. 6 and 9). Responses to a given chemical at suprathreshold concentration were averaged, and a paired t-test compared the average firing rate before the chemical with the average firing rate at 1-s intervals after chemical application. There was a degree of variability in absolute response magnitude across neurons. Therefore the population dose-response data, and mean responses across application trials, for each chemical were subjected to a nonparametric van der Waerden analysis of overall treatment effects, followed by post hoc comparisons among treatment levels: P < 0.05 was accepted as significant.

We investigated the effect of the H1 antagonist (tongue units only) and the nicotinic antagonist (tongue and corneal-conjunctival units) as follows. To test the effect of the H1 antagonist, histamine (10%) was delivered to the tongue at least three times at 5-min interstimulus intervals. Thirty seconds before the next scheduled histamine stimulus, cetirizine was delivered to the tongue in an identical manner. Histamine continued to be delivered at the 5-min interstimulus interval to evaluate the time course of any effect of cetirizine on histamine-evoked responses. A similar paradigm was followed to determine the effect of mecamylamine on nicotine-evoked responses of tongue or corneal-conjunctival units. In some cases, the H1 antagonist was tested similarly against nicotine-evoked responses and mecamylamine against histamine-evoked responses. Data were pooled and mean responses before and after application of the antagonist were compared using a paired t-test.

For experiments in which capsaicin was tested on tongue units, we usually observed an increase in spontaneous activity after application of capsaicin at a suprathreshold concentration. In these cases, we recorded the spontaneous firing for ≈1 h after the capsaicin stimulus. Data were pooled and mean spontaneous firing levels at different times postcapsaicin were compared with the precapsaicin baseline level using a paired t-test.

Histology

At the conclusion of successful recordings, an electrolytic lesion was made at the recording site by passing current (6 V DC) through the microelectrode. In the few experiments using carbon-fiber microelectrodes, the exact location of the electrode penetration was noted, a tungsten microelectrode was inserted at that site to the same depth, and an electrolytic lesion was made. At the conclusion of the experiment, the animal was killed by overdose of thiopental, and the brain stem removed and postfixed in 10% formalin. The brain stems were cut in 50-μm frozen sections, collected on glass slides, counterstained, and examined under the light microscope. Sections containing the lesion were drawn by camera lucida. Lesion sites were collectively plotted onto representative brain stem sections (Fig. 1).

RESULTS

Unit sample

TONGUE UNITS. Each of the 32 units responded to mechanical (pressure-pinch) stimuli and noxious heating of the tongue, 60% responded to cooling, and all but one responded additionally with a clear increase in firing rate to at least one chemical (Table 1). The majority of units exhibited low (<1–2 Hz) or no background firing, whereas the remainder were spontaneously active at rates >8 Hz (maximum 500 imp/60 s). The mean spontaneous firing rates for all tested units are plotted in Fig. 6. Mechanically sensitive receptive fields almost always included the tip of the tongue as well as more posterior and lateral areas of the tongue ipsilaterally. Responses usually appeared to be evoked by pinching the tip of the tongue bilaterally, although it was difficult to ascertain the precise extent of the mechanical receptive fields because the tongue was not stabilized. The majority of units (55%) had mechanical receptive fields solely on the tongue, whereas the remainder responded additionally to pressure or pinch stimuli delivered to the lower lip and/or point of the chin ipsilaterally. One unit additionally responded to pinching the corner of the mouth. Most units appeared to respond to bilateral stimulation of the tip of the tongue when it was within the receptive field (Figs. 2 and 10A). In general, units with receptive fields including tongue and chin tended to be located caudal to those with input only from the tongue. Eighty-one percent (26/32) of the units were categorized as WDR because they responded to noxious mechanical stimuli and/or nocuous cooling of the tongue as well as noxious heat, whereas the remainder (19%) were categorized as nociceptive-specific because they responded only to
stimuli, were needed to evoke a response. In contrast, von Frey thresholds for evoking responses from the eyelid were extremely low (<1 mN). The units with larger facial receptive fields responded to blowing or light brushing of fur as well as to noxious cutaneous pin-prick stimuli. A majority of units tested (19/28) responded to cooling of the cornea-conjunctiva. In addition to their responses to mechanical and cooling stimuli, all units responded to noxious heat and were thus classified as WDR.

Eighteen of the units did not exhibit any spontaneous firing, while the remainder fired spontaneously. Ten units fired spontaneously at a low rate (<1 Hz), whereas 3 fired at rates of 3–10 Hz.

### Unit recording sites

**TONGUE UNITS.** Recording sites were histologically recovered in most (84%) experiments, and are shown in Fig. 1 (●). Virtually all were located in the most superficial layer of the dorsomedial trigeminal nucleus caudalis, ipsilateral to the receptive field, in a distribution indistinguishable from that of c-fos-immunoreactive cell nuclei after noxious chemical stimulation of the tongue (Carstens et al. 1995).

**CORNEAL-CONJUNCTIVAL UNITS.** Recording sites were histologically recovered for 25 units and are shown in Fig. 1 (▲). Four were located superficially, and the remainder were located in deeper layers of the ventrolateral trigeminal nucleus caudalis, ipsilateral to the unit’s receptive field.

### Responses to different noxious chemicals

**TONGUE UNITS.** All but one unit responded to application of at least one noxious chemical to the tongue, and most units responded to several different chemicals (Table 1).

Overall, the 32 units responded to 75.3% of the different chemicals tested, and nearly one-third responded to all chemicals (<10) applied. Thus 87.5% of the units tested responded to histamine, 81% to nicotine, 74% to NaCl, 61% to 5-HT, 89% to acid, 80% to ethanol, 75% to capsaicin, 55.5% to piperine, and 50% to mustard oil. The percentages for piperine and mustard oil are probably an underrepresentation because these chemicals were almost always tested after prior application of capsaicin [Table 1, (−)], which frequently desensitized the tongue (see further text).

Unit responses to application of a given chemical were not markedly affected by prior application of a different chemical, except for capsaicin, piperine, and mustard oil, which often appeared to desensitize the tongue. We determined the number of chemicals evoking responses in neurons that were grouped according to the chemical that they were first exposed to. The 22 units tested first with histamine subsequently responded to 82.4% of up to eight additional chemicals tested. Similarly, four units tested first with 5-HT subsequently responded to 88.4%, three with NaCl to 73.7%, and two with acid to 85.7% of the additional chemicals tested. One unit tested initially with nicotine responded to both of two additional chemicals.

An example of one unit’s responses to a variety of different chemicals is shown in Fig. 2. Each row shows peristimulus time histograms (PSTHs) of the unit’s responses to a given chemical, arranged from left to right by increasing...
TABLE 1. Incidence of individual dorsomedial caudalis (tongue) unit responses to application of various chemicals to the tongue

<table>
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<tr>
<th>Unit</th>
<th>Cold</th>
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<th>5-HT</th>
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Sum 18/30 28/32 22/27 11/18 17/23 16/18 12/15 15/20 5/9 8/16
Total 134/178

+, excited; –, not excited; (−), not excited after capsaicin, piperine, or mustard oil; open space, not tested. His, histamine; nic, nicotine; 5-HT, serotonin; pH, acidified phosphate buffer; EtOH, ethanol; cap, capsaicin; pip, piperine; and MO, mustard oil.

*Concentration (1st 3 PSTHs in row) and by repeated trials at one concentration (last 3 PSTHs in row). The bottom row shows, from left to right PSTH, the unit’s responses to physical stimuli, piperine, and carbonated water. This unit had a receptive field on the ipsilateral tip of the tongue and chin (Fig. 2, bottom left inset) and responded strongly to noxious heating of the tongue but only weakly to pressure and did not appear to respond appreciably to cooling (Fig. 2, bottom left). Most importantly, this unit responded to each chemical tested, as indicated by the middle column of PSTHs. Responses evoked by each of the chemicals were of comparable magnitude, but response duration appeared to be briefer for histamine, nicotine, ethanol, and carbonated water compared with the other chemicals. Furthermore, response magnitude increased in a dose-related manner for each chemical tested (1st 3 PSTHs in rows 1–7). Successive responses to repeated suprathreshold applications of 5-HT, nicotine, capsaicin, and mustard oil decreased markedly across trials (3 righthand PSTHs in rows 1, 4, 7, and 8, respectively), whereas successive responses to the other chemicals decreased less or not at all. The unit’s recording site was in dorsomedial caudalis (Fig. 2, bottom right inset).

Corneal-Conjunctival Units. Most of these units responded to a majority or all of the tested chemicals, whereas only two were unresponsive (Table 2). Overall, the 31 units responded to 65% of up to nine chemicals tested per unit (116/179 chemical stimulus applications). Table 2 provides an overview of unit responsiveness to the various chemicals. The percentages of units responding to each chemical are as follows: nicotine (88%), capsaicin (82%), EtOH (79%), acid (70%), NaCl (67%), piperine (61.5%), mustard oil (58%), histamine (46%), and 5-HT (25%). An individual example of a unit’s responses to various chemicals applied to the cornea-conjunctiva is shown in Fig. 3. This unit had a cutaneous receptive field that encompassed the eye and part of the lateral face (bottom left inset, hatched) and responded to noxious heat and innocuous cold and pressure stimuli applied to the ocular surface (Fig. 3, bottom left PSTH). The PSTHs in the middle column and in the bottom row show the responses of this unit to application of each of the nine chemicals tested. Note that this unit usually gave a brief high-frequency discharge when the chemical was applied (at arrows), and again 60 s later when the saline rinse was applied; this represents a response to mechanical stimulation by the fluid drop. The chemically evoked response occurred after the initial mechanical response. This unit gave the largest responses to histamine, nicotine, NaCl (1st 3 rows), ethanol (5th row), and mustard oil (middle PSTH in bottom row). The three left-hand columns of PSTHs in the first three rows show that the unit’s
responses increased with concentration of histamine, nicotine, and NaCl. The three right-hand columns show that the unit’s responses generally declined on repeated application of most of the chemicals, with the exception of NaCl (3rd row).

The order in which the various chemicals were presented did not appear to influence whether the unit responded to subsequent chemicals, with the exception of capsaicin. Thus the 8 units tested first with histamine responded to 75.9% of the additionally tested chemicals, 10 tested first with acid responded to 78.6%, 6 tested first with NaCl responded to 71.4%, and 1 tested first with nicotine responded to 100%, of additionally tested chemicals. The three units tested first with 5-HT responded to only 31.8% of the additionally tested
TABLE 2. Incidence of individual ventrolateral caudalis (corneal-conjunctival) unit responses to application of various chemicals to cornea-conjunctiva

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Sum 11/24 21/24 4/16 16/24 16/24 11/14 18/22 8/13 11/19
Total 116/179

+, excited; −, unresponsive; (−), unresponsive after capsaicin; open: not tested. His, histamine; nic, nicotine; 5-HT, serotonin; pH, acidified phosphate buffer; EtOH, ethanol; cap, capsaicin; pip, piperine; and MO, mustard oil.

chemicals; two of these units were also unresponsive to 5-HT. The one unit tested initially with capsaicin did not respond to any of five subsequently presented chemicals (Table 2).

Time Course of Chemically Evoked Responses

TONGUE UNITS. To compare the temporal profile of unit responses to the application of different chemicals to the tongue, the initial response of each unit to each chemical at a suprathreshold concentration was selected. Responses to each chemical were averaged across units and are shown in Fig. 4 aligned with stimulus application (→). The averaged responses increased significantly within 1–5 s after application of each chemical except ethanol (Fig. 4), although there were apparent differences in the time courses. Responses to histamine and 5-HT achieved a maximal firing rate most rapidly, whereas responses to nicotine, NaCl, ethanol, acid (pH), capsaicin, and mustard oil built up more slowly to the peak response (Table 3). The response to 5-HT was biphasic because some units gave a rapid and brief response, whereas others gave a slower and more prolonged response (Fig. 4). Peak firing rates were approximately equivalent for each chemical (Table 3). After the peak firing rate was achieved, responses declined during the 60-s period (Fig. 4) but remained significantly elevated throughout for all chemicals except histamine, nicotine, and mustard oil. The mean response evoked by histamine declined most quickly and was no longer significantly elevated 10 s after application. The mean nicotine-evoked response was no longer significantly elevated after 34 s, and the mean response to mustard oil was no longer significantly elevated after 30 s.

CORNEAL-CONJUNCTIVAL UNITS. Averaged responses of these units to eight different chemicals are shown in Fig. 5. The brief initial peaks coincident with chemical application (Fig. 5, →) represent the mechanical response component that was apparent in approximately one-half of the units. For each chemical, there was an increase in the mean firing rate after the mechanical response component. Peak firing rates were reached most quickly (Table 3), and declined most rapidly (Fig. 5), with histamine, nicotine, and ethanol. The peak response took longer to build up with NaCl, acid, and mustard oil (Table 3) and declined more slowly; firing rates were still significantly elevated 60 s later (Fig. 5). Response profiles for capsaicin and acid (pH) were both characterized by a steady elevation in firing rate that persisted throughout the 60-s stimulus period (Fig. 5). Highest, and approximately equivalent, mean maximal firing rates were achieved after nicotine, ethanol, NaCl, and mustard oil, whereas 5-HT and piperine were least effective (Table 3; Fig. 5).
Dose-response relationship and tachyphylaxis

TONGUE UNITS. Histamine. Most units responded to application of histamine to the tongue only at higher concentrations (1, 10%). Figure 6A shows individual dose-response curves for 20 units (thin lines) as well as the mean dose-response curve (thick line with error bars). The spontaneous activity (SA) level for each unit also is plotted. The treatment (dose) effect was significant ($F = 27.09, P = 0.0001$). Posthoc comparison revealed that the response to 10% histamine was significantly greater than that to 1% (Fig. 6A, *).

Figure 6B plots individual unit responses across histamine application trials repeated at 5-min interstimulus intervals. A few units exhibited declining or increasing responses, whereas most were fairly constant with no significant change across trials.

Nicotine. Figure 6C plots responses versus nicotine concentration for 16 units. Overall, responses increased significantly with dose of nicotine ($F = 31.24, P = 0.0001$), with responses to 1 and 10% significantly greater compared with lower doses. Figure 6D plots responses versus nicotine application trial. Mean responses significantly declined across trials ($F = 41.7, P = 0.0001$), with the mean response to trial 2 significantly lower compared with trial 1, and the mean response to trial 3 significantly lower compared with trial 2. Responses to trials 2 and 3 were 74 and 59.2% of trial 1, respectively.

NaCl. Unit responses increased significantly as a function of NaCl concentration (Fig. 6E; $F = 13.79, P = 0.0001$), with the mean response at 5 M being significantly larger compared with lower concentrations. In Fig. 6F, it can be seen that some units’ responses declined over repeated trials of NaCl application, whereas many remained constant. On average, however, the declines at trial 2 and trial 3 (84.7 and 77.7% of trial 1, respectively) were not significant.

Ethanol. Unit responses increased significantly as a function of ethanol (EtOH) concentration (Fig. 6G; $F = 23.79, P = 0.0001$) with the response at 25% ethanol significantly larger compared with lower concentrations. Figure 6H shows that on average, responses to ethanol application did not
FIG. 4. Time course of responses of dorsomedial caudalis units to application of different chemicals to the tongue. Shown are averaged PSTHs (binwidth: 1 s) of group responses to application of each of the indicated chemicals at a suprathreshold concentration (1 or 10% for histamine, 1 or 10% for nicotine, 2.5 or 5 M for NaCl, 25 or 50% for EtOH, 3% for 5-HT, pH 1–3 for acid, 0.001–0.1% for capsaicin, and 7.5–25% for mustard oil). Numbers above PSTHs indicate number of units. Error bars: SD; * Significantly different from mean response before chemical stimulus application ($P < 0.05$, paired $t$-test).

change significantly across trials (mean response, trial 3 = 83% of trial 1).

Responses evoked by ethanol cannot be attributed solely to evaporative cooling of the tongue because four units that responded to ethanol did not respond to cooling the tongue and one unit unresponsive to ethanol did respond to tongue cooling (Table 1). Cooling may have contributed to the response evoked by ethanol in the seven units that responded to both.

5-HT. Mean responses increased significantly as a function of 5-HT concentration (Fig. 6I; $F = 8.44$, $P = 0.0057$) with the response to 5% significantly larger than the spontaneous rate. Figure 6J plots responses across application trials. The mean response to the second trial was significantly lower (62.5%) compared with the first trial. The mean response to the third trial was not significantly different compared with the first trial.

Acid ($pH$). Although the mean dose-response curve (Fig. 6K) was fairly flat, there was a significant effect ($F = 21$, $P = 0.0001$) with the mean response at pH 1 being significantly larger compared with responses at higher pH values. Figure 6L shows that responses of most units were fairly consistent across trials, with no significant change in mean responses.

Capsaicin. The determination of dose-response relationships with capsaicin proved to be difficult because the first
effective dose of capsaicin often resulted in an apparent desensitization. Although many units exhibited an increased response to a suprathreshold versus subthreshold dose of capsaicin (e.g., 0.001% vs. 0.01% or 0.01% vs. 0.1%; Fig. 6M), subsequent responses to even higher concentrations of capsaicin were usually smaller than the initial response. This is reflected in the mean dose-response curve in Fig. 6M, which nonetheless demonstrated a significant dose effect (F = 16.26, P = 0.0001) in which the response to 0.01% capsaicin was significantly larger compared with the lower dose. Figure 6N shows that responses to subsequent applications of capsaicin declined significantly (F = 30.7, P = 0.0001). The response to trial 2 was 46.4% of the initial response.

After application of a suprathreshold concentration of capsaicin, units often became unresponsive to other chemicals as well. Thus 6 of 12 units were unresponsive to mustard oil, 4 of 9 were unresponsive to piperine, 2 of 2 were unresponsive to acid at pH 1, 2 of 3 were unresponsive to histamine, and 1 of 1 was unresponsive to nicotine after prior application of capsaicin. One unit that did not respond to capsaicin had received prior mustard oil.

A marked increase in spontaneous activity after initial application of capsaicin was observed in most units tested and is illustrated in Fig. 7A. The left PSTH shows a unit’s low spontaneous firing level before and after initial application of capsaicin. The two middle PSTHs in Fig. 7A show dramatic increases in spontaneous firing 8 and 15 min after application of capsaicin. The spontaneous level had decreased but had not reattained the precapsaicin level, after 1 h (Fig. 7A, right PSTH). Typically, spontaneous firing waxed and waned in frequency as evident in Fig. 7A. The spontaneous firing rate at various times following the initial application of capsaicin was averaged in five units (Fig. 7B). It was significantly higher 4–6 min after the initial application of capsaicin (P = 0.0224, paired t-test) compared with the precapsaicin level and declined during the next hour (Fig. 7B).

**Piperine.** Figure 6O shows that responses of the three units tested increased from 0.1 to 1% piperine although the sample size was insufficient for statistical testing. Mean responses significantly decreased across trials (Fig. 6P; F = 99.7, P = 0.0001) with the mean response to the second application being significantly lower (46%) compared with the initial response.

**Mustard oil.** Figure 6Q shows that responses tended to increase with mustard oil concentration in four units tested, and Fig. 6R shows that responses declined over repeated application trials in three units but first increased in a fourth unit.

**Carbonated water.** Each of four units responded to application of fresh carbonated water to the tongue. An example is shown in Fig. 8A. The unit did not respond to application of phosphate buffer at pH 6 (Fig. 8A, right PSTH), indicating that excitation by the carbonated water (pH = 6.1) was not due solely to pH. Figure 8B shows that three units gave fairly reproducible responses while responses of one declined over repeated application trials.

**CORNEAL-CONJUNCTIVAL UNITS.** **Histamine.** Figure 9A shows dose-response curves for responses of corneal-conjunctival units to histamine. The dose effect was significant (F = 13.2, P = 0.0001), with the mean response to 10% histamine being significantly larger compared with lower concentrations. Figure 9B shows responses to repeated trials of histamine application at one supramaximal concentration (10%). The overall effect was significant (F = 41.8, P = 0.0001) with the mean response to the second application trial being significantly smaller compared with the first and third trials.

**Nicotine.** Mean responses increased significantly with nicotine dose (Fig. 9C; F = 36.8, P = 0.0001); the mean response to 10% was significantly greater than to 1%, and the mean response to 1% was greater than to 0.1%. Mean responses declined significantly across repeated trials of nicotine (Fig. 9D; F = 15.9, P = 0.0001), with the mean responses to trials 2 and 3 being significantly smaller compared with trial 1.

### Table 3. Parameters of mean responses of tongue and corneal-conjunctival units to chemical stimuli

<table>
<thead>
<tr>
<th>Chemical</th>
<th>n</th>
<th>Time to Peak Firing, s</th>
<th>Mean Peak Rate, Hz</th>
<th>Range of Peak Firing Rate, Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tongue</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histamine</td>
<td>22</td>
<td>10.2 ± 12.2</td>
<td>23.4 ± 18.2</td>
<td>6–61</td>
</tr>
<tr>
<td>Nicotine</td>
<td>19</td>
<td>14.8 ± 10.3</td>
<td>22.2 ± 17.4</td>
<td>6–67</td>
</tr>
<tr>
<td>NaCl</td>
<td>15</td>
<td>17.7 ± 10.5</td>
<td>20.2 ± 17.8</td>
<td>4–63</td>
</tr>
<tr>
<td>Ethanol</td>
<td>11</td>
<td>16.8 ± 7.9</td>
<td>23.7 ± 23.2</td>
<td>5–82</td>
</tr>
<tr>
<td>5-HT</td>
<td>10</td>
<td>10.9 ± 5.5</td>
<td>16.1 ± 7</td>
<td>7–29</td>
</tr>
<tr>
<td>Acid</td>
<td>15</td>
<td>16.2 ± 9.5</td>
<td>23.3 ± 16.9</td>
<td>4–56</td>
</tr>
<tr>
<td>Capsaicin</td>
<td>12</td>
<td>23.9 ± 8.8</td>
<td>17.5 ± 16.3</td>
<td>4–58</td>
</tr>
<tr>
<td>Mustard Oil</td>
<td>8</td>
<td>16.6 ± 8.4</td>
<td>20.6 ± 19.9</td>
<td>7–68</td>
</tr>
<tr>
<td><strong>Cornea-conjunctiva</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histamine</td>
<td>10</td>
<td>12.2 ± 10.4</td>
<td>16.1 ± 10.3</td>
<td>5–33</td>
</tr>
<tr>
<td>Nicotine</td>
<td>20</td>
<td>13.8 ± 1</td>
<td>27.9 ± 21.7</td>
<td>5–77</td>
</tr>
<tr>
<td>NaCl</td>
<td>15</td>
<td>19.6 ± 9.1</td>
<td>32 ± 15.9</td>
<td>12–52</td>
</tr>
<tr>
<td>Ethanol</td>
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<td>9.5 ± 6.6</td>
<td>34.3 ± 28.8</td>
<td>10–95</td>
</tr>
<tr>
<td>5-HT</td>
<td>4</td>
<td>11.8 ± 6.3</td>
<td>10.3 ± 10.6</td>
<td>4–26</td>
</tr>
<tr>
<td>Acid</td>
<td>14</td>
<td>19.6 ± 11.1</td>
<td>20.5 ± 15.7</td>
<td>8–46</td>
</tr>
<tr>
<td>Capsaicin</td>
<td>16</td>
<td>28.3 ± 10.4</td>
<td>19.5 ± 18.3</td>
<td>5–54</td>
</tr>
<tr>
<td>Mustard Oil</td>
<td>12</td>
<td>14.4 ± 5.6</td>
<td>25.6 ± 19.8</td>
<td>7–55</td>
</tr>
<tr>
<td>Piperine</td>
<td>6</td>
<td>21.8 ± 13.6</td>
<td>9.8 ± 6.9</td>
<td>5–23</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, number of units.
**NaCl.** Mean responses increased significantly with NaCl dose (Fig. 9E; $F = 178, P = 0.0001$) with the response to 5 M NaCl greater than to lower doses, and the response to 2.5 M NaCl greater than the spontaneous level. Responses to repeated application of NaCl did not change significantly across trials (Fig. 9F).

**Ethanol.** Mean responses increased significantly with increasing ethanol concentration (Fig. 9G; $F = 7.32, P = 0.0006$) with the response to 50% ethanol being significantly greater than the spontaneous level. Mean responses to repeated application of ethanol decreased significantly (Fig. 9H; $F = 58.4, P = 0.0001$) with the responses to the second and third trials being significantly lower compared with the first trial.

Of 10 units tested with both ethanol and cooling stimuli, 6 units were activated by both cooling and ethanol, 2 were excited by ethanol but not cooling, and 2 were excited by cooling but not ethanol. These data indicate that unit responses to ethanol were not due exclusively to evaporative cooling.

**5-HT.** Our sample of units was fairly unresponsive to 5-HT. Dose-response relationship was determined for only one unit (Fig. 9I), and responses of 2 of 4 units decreased with repeated 5-HT application (Fig. 9J). The sample size was too small for statistical analysis.
Acid (pH). There was considerable variability in unit responses to acidified buffer. For this reason, units were divided into groups giving maximal responses of either <200 (Fig. 9K) or >200 imp/60 s (Fig. 9L). Responses of most units tended to increase with decreasing pH, and the dose effect was significant for all units pooled (F = 5.5, P = 0.0012).

For the group exhibiting lower firing rates (Fig. 9K), the mean response to acid at pH 1 was significantly larger compared with the spontaneous firing rate. However, a number of units that responded to acid at pH 3 or 4 gave smaller responses at lower pH levels (Fig. 9, K and L). When acidified buffer at a constant pH of 1 was applied repeatedly, successive responses declined significantly (Fig. 9M; F = 15.9, P = 0.0001) with the response to the third trial being significantly lower compared with the first trial.

Capsaicin. Mean responses significantly increased with capsaicin dose (Fig. 9N; F = 12.63, P = 0.0001) with the response to 0.1% capsaicin being significantly larger than to lower doses. Figure 9O shows that the responses of all six units tested decreased from the first to second application of capsaicin and this effect was significant (F = 31.38, P = 0.0001).

An increase in the spontaneous firing rate was noted in five units after initial application of capsaicin to the eye, but this was not systematically investigated further. We also did not systematically investigate possible cross-desensitization effects of capsaicin on responses evoked by subsequent application of other chemicals to the eye. After application of capsaicin, 9 of 15 units responded to mustard oil and 7 of 11 responded to piperine. However, because these latter chemicals were only rarely tested without prior application of capsaicin, we cannot be certain if the incidence of responsiveness to mustard oil and piperine was reduced by prior capsaicin.

Mustard oil. Mean responses increased significantly as a function of mustard oil concentration (Fig. 9P; F = 9, P = 0.006), with the mean response to 50% mustard oil being significantly larger compared with the spontaneous level. In Fig. 9Q, responses of 8 of 10 units decreased across trials of repeated mustard oil application although the overall change was not significant.

Piperine. Figure 9R shows that the responses of the three units tested increased with concentration of piperine. The responses of all three units decreased over repeated trials of piperine application although the responses of two first increased from trial 1 to trial 2 (Fig. 9S).

Antagonists

TONGUE UNITS. Many caudalis units responded to different classes of irritant chemicals. We wished to determine if the response to a particular chemical was mediated by a transduction mechanism specific to that chemical. Although transduction mechanisms for some of the presently tested chemicals are not known, responses to histamine and nicotine presumably are mediated by H1 and neuronal nicotinic receptors, respectively. We therefore investigated if an H1 antagonist, ceterizine, or the nicotinic ganglionic blocker, mecamylamine, could reduce or abolish the excitatory effect of histamine or nicotine, respectively.

Figure 10A shows responses of a dorsomedial caudalis unit to application of histamine to the tongue, before (Fig. 10A, top left) and 30 s after topical application of ceterizine to the tongue (Fig. 10A, top middle). Immediately after ceterizine the response to histamine was markedly reduced. Five minutes later the histamine response had fully recovered (Fig. 10A, top right). Figure 10A, middle, shows that identical application of ceterizine had no effect on the same unit’s responses to nicotine. As shown in Fig. 10A, bottom, the response to nicotine (left) was reduced markedly after application of mecamylamine to the tongue (middle); the nicotine response had recovered partly 1 h later (right). Figure 10B shows data from another unit in which mecamylamine markedly reduced the response evoked by application of nicotine to the tongue (top middle), with little recovery 20 min later (top right), whereas mecamylamine did not reduce the same unit’s response to histamine (Fig. 10B, bottom). Application of ceterizine or mecamylamine alone did not evoke responses in any units.

Responses to application of histamine to the tongue were significantly attenuated (to 47.5%; P = 0.033, paired t-test) after ceterizine (1%) in each of 10 units tested. Responses subsequently recovered to the control level within 5 min in 9 of 10 units, whereas in 1 unit the histamine-evoked response was suppressed only after a delay of 15 min after application of ceterizine. A lower dose of ceterizine (0.1%) also reduced responses to histamine in 2 of 3 units tested.

Responses to application of nicotine to the tongue were also significantly attenuated (to 33%, P < 0.05, paired t-test) 30 s after mecamylamine in four units. Partial recovery of the response to nicotine was only observed in 2 of 4 units during the next 30–60 min.

CORNEAL-CONJUNCTIVAL UNITS. The mean response evoked by application of nicotine to the eye was significantly attenuated (to 65%; P = 0.004, paired t-test) immediately after application of mecamylamine (0.1%) in four units with full recovery 5 min later. An example is shown in Fig. 11. The histamine antagonist was not tested with corneal-conjunctival units because they exhibited a lower incidence of responsiveness to histamine (Table 2).

DISCUSSION

These results have identified a population of WDR and nociceptive-specific neurons in the superficial laminae of the dorsomedial aspect of trigeminal subnucleus caudalis that was activated by irritant chemical stimulation of the tongue, as well as a second population of WDR neurons in ventrolateral caudalis that was also activated by irritant chemical stimulation of the cornea-conjunctiva. A salient finding is that most caudalis neurons responded to application of a broad spectrum of irritant chemicals to the tongue or ocular mucosa (Tables 1 and 2). Furthermore, responses to histamine and nicotine were reduced or prevented by prior application of H1 or nicotinic antagonists, respectively. Therefore our data indicate that a substantial fraction of trigeminal caudalis units are activated by multiple irritant chemicals at least partly via separate peripheral transduction mechanisms. These findings are discussed further in relation to previous work and to the neural coding of oral irritation.
Chemically evoked responses and other properties of tongue and corneal-conjunctival units

Most tongue and corneal-conjunctival units responded to one or more of the chemicals tested. The incidence of both tongue and corneal-conjunctival units’ responsiveness to individual chemicals was generally similar except that corneal-conjunctival units were notably less responsive to histamine compared with sensory fibers within the lingual epithelium. (°1 mM) was reported to have no effect on chemosensitive corneal afferents (MacIver and Tanelian 1993a). Both of the presently studied unit populations displayed prolonged responses to capsaicin and acid (Figs. 4 and 5). The maximal firing rates of corneal-conjunctival units in response to nicotine, NaCl, ethanol, and mustard oil were, on average, 5–12 Hz higher compared with those of tongue units (Table 3). This might be because corneal nociceptor terminals that reach to within 5 μm of the epithelial surface (MacIver and Tanelian 1993a,b) may be more accessible to chemicals compared with sensory fibers within the lingual epithelium.
The present experiments directly tested for tachyphylaxis or sensitization of successive unit responses to repeated application of chemicals at a 5-min interstimulus interval. This interval was selected as a compromise to test both for prolonged effects of a given chemical and to allow several chemicals to be tested sequentially. Tongue units exhibited significant tachyphylaxis to nicotine, 5-HT, and capsaicin (Fig. 6), whereas corneal-conjunctival units showed significant tachyphylaxis to histamine, nicotine, ethanol, acid, and capsaicin (Fig. 9). None of the chemicals evoked successively increasing responses indicative of a sensitizing effect. If any of the chemicals did elicit sensitization, this effect presumably was counteracted by an equal or stronger tachyphylaxis at the 5-min interstimulus interval used. It is conceivable that longer-lasting changes such as central sensitization may have resulted from chemical application. This could produce a rise in neuronal excitability leading to enhanced firing over a time course of hours that might not be detected using a 5-min interstimulus interval. Although we cannot exclude this possibility, we did not observe any cases in which a unit’s responses to successively tested chemicals showed a marked increase in firing rate over time.

Although capsaicin appeared to induce cross-tachyphylaxis to subsequently tested chemicals, other chemicals did not appear to do so. Even nicotine, which exhibited the most consistent tachyphylaxis in both unit populations, did not show cross-tachyphylaxis in the few cases tested although this requires further verification. Therefore the data indicate that unit responses were not markedly influenced by prior chemicals except capsaicin.

Although all corneal-conjunctival units had ipsilateral cutaneous receptive fields, most of the tongue units appeared
FIG. 7. Increase in spontaneous firing after initial capsaicin application. A: PSTHs show an individual unit’s initial low firing rate immediately after application of capsaicin (left PSTH), followed by a progressive increase in spontaneous firing 8 and 15 min after capsaicin (middle PSTHs), which declined after 1 h (right PSTH). Note also waxing and waning of spontaneous rate. B: graph plots mean spontaneous activity (60 s epochs) before (pre), immediately after application of capsaicin (cap), and at progressively later time periods after initial capsaicin. * Significantly larger than precap response ($P < 0.05$, paired t-test).

to respond to bilateral mechanical stimulation of the tip of the tongue when this was within the cutaneous receptive field. Such units might receive bilateral input from lingual afferents. Previous anatomic tracing studies have shown bilateral trigeminal primary afferent projections to caudalis from midline structures such as tongue or nose but not from the cornea (Arvidsson and Gobel 1981; Jacquin et al. 1983, 1990; Marfurt 1981; Marfurt and Rajchert 1991; Pfaller and Arvidsson 1988; Torvik 1956). A recent study has shown a bilateral distribution of c-fos–immunoreactive neurons in dorsomedial caudalis after unilateral chemical stimulation of the dorsal anterior tongue (Carstens et al. 1995).

Comparison with previous studies

There are relatively few previous studies of responses of trigeminal neurons to noxious chemical stimuli. Mosso and Kruger (1973) reported a small number of caudalis units to respond to application of 0.1 M acetic acid to the cornea, as confirmed presently. Caudalis units with intraoral C-fiber afferent input responded to noxious levels of CO$_2$ and mustard oil vapor (Peppel and Anton 1993), and units in oralis and caudalis with facial receptive fields responded to intracutaneous injection of formalin (Raboisson et al. 1991, 1995). Mustard oil given intramuscularly or topically to skin resulted in an expansion of mechanoreceptive fields of caudalis units (Hu et al. 1992; Yu et al. 1993). Most relevant to the present tongue units is Amano et al.’s study (1986), which shows that a substantial fraction of WDR and nociceptive specific-type units in trigeminal caudalis in cat receiving muscle afferent input could be excited by intraarterial injection of up to five irritant chemicals [7% NaCl, KCl (3.6 mg/0.6 ml), bradykinin (25 μg/0.5 ml), serotonin (84 mg/0.5 ml), histamine (50 mg/0.5 ml)] into the lingual artery supplying tongue muscle or into the arterial supply of a jaw muscle. Most responsive units were located in deeper laminae of caudalis although a few were in the superficial lamina, where all of the present tongue units were located. Mean response latencies after intraarterial injection were short (<2 s) for NaCl and KCl and were 10, 7, and 3.3 s for histamine, bradykinin, and serotonin, respectively. In the present study, response latencies to application of all chemicals except ethanol to the tongue were similarly short (<5 s; Fig. 5) and presumably reflect the time of chemical diffusion to nociceptor terminals in more superficial layers of the mucosal epithelium. It is conceivable that more lipid-soluble chemicals such as capsaicin may have diffused into muscle to additionally activate intramuscular nociceptors. All of the present tongue units responded to squeezing and noxious heat; we did not determine if they received direct muscle afferent input. Thus our data and those of Amano et al. (1986) indicate that a substantial fraction of nociceptive

FIG. 8. Response to carbonated water. A: response of caudalis unit to carbonated water (left PSTH) and lack of response to phosphate buffer fixed at pH 6 (right PSTH). Inset: receptive field (black) on ipsilateral tongue and chin. B: graph as in Fig. 5 (right) plotting individual (thin lines) and mean (thick line) unit responses to repeated application of carbonated water (interstimulus interval: 5 min).
units in caudalis can respond to multiple irritant chemicals given topically or intramuscularly to the tongue.

Several previous studies have described the properties of trigeminal caudalis units with afferent input from the cornea in the rat (Meng et al. 1997; Nagano et al. 1975; Pozo and Cervero 1993) and cat (Mosso and Kruger 1973; Nishida and Yokota 1991). Most relevant are two recent studies investigating the properties of units in superficial locations of the ventrolateral caudalis corresponding to the regions of termination of corneal afferents (Meng et al. 1997; Pozo and Cervero 1993). Pozo and Cervero (1993) sampled units in superficial laminae at the caudalis-interpolaris transition area (+0.5-mm rostral to −1 mm-caudal to obex) that responded to electrical stimulation of the cornea. Units had mechanical receptive fields that were either restricted to the cornea or included the cornea and surrounding skin. They were categorized as class 2, responding to noxious and non-noxious mechanical stimulation of the cornea, or class 3, responding only to noxious corneal stimuli. Most units displayed no spontaneous activity. Each of seven units tested also responded to noxious heating of the cornea, similar to our present unit sample. Meng et al. (1997) have recorded from WDR and nociceptive-specific units located ventrolaterally at the junction of caudalis and the C1 spinal cord and at the caudalis-interpolaris transition region. A majority of units at the caudalis-interpolaris transition that were electrically driven from the cornea had no receptive field; of the remainder, the majority responded only to low-threshold mechanical stimuli, whereas 25% were WDR type. All units at the caudalis-C1 junction had receptive fields on the cornea and periorbital skin and were classified as WDR type or nociceptive specific. Most units displayed little or no spontaneous activity, and nearly all WDR units at the caudalis-interpolaris transition, as well as units at the caudalis-C1 junction, responded to noxious thermal stimulation as well as mustard oil applied to the cornea. Our results confirm and extend these latter findings by showing that a large fraction of caudalis WDR units responded to application of a variety of additional irritant chemicals to the eye. Because chemicals were applied by instillation into the eye, we cannot determine if responses were mediated by activation of chemosensitive nociceptors supplying the cornea, conjunctiva, or both.

**Diversity of irritant chemicals**

Many chemicals elicit a sensation of irritation when applied to oral or ocular mucosa or skin, including capsaicin in red peppers (Dupuy et al. 1988; Green 1989, 1991; Karrer and Bartoshuk 1991), piperine in black pepper (Lawless and Stevens 1990), nicotine in tobacco (Dessirier et al. 1997), menthol in black pepper (Liu et al. 1993), citric acid (Liu and Simon 1996a; Liu et al. 1993; Sucher et al. 1990) and atropine, and, in a few instances, alpha-bungarotoxin (Liu et al. 1993), suggesting involvement of multiple subtypes of the neuronal nicotinic receptor (Deneris et al. 1991; Ochoa et al. 1990; Sargent 1993). The present data, showing nicotinic activation of a large fraction of tongue units in a mecamylamine-sensitive manner, are consistent with the idea that nicotine activates lingual nociceptors via a neuronal nicotinic receptor.

There was a significant tachyphylaxis in responses of both tongue and corneal-conjunctival units to repeated nicotine (Figs. 6D and 9D), which corresponds well with recent psychophysical data from our laboratory showing a significant decline in ratings of irritation to repeated application of nicotine to the tongue (Dessirier et al. 1997). Furthermore, irritation from nicotine was cross-desensitized by capsaicin (Dessirier et al. 1997). Capsaicin abolished the response to nicotine in the one caudalis unit tested presently. These data corroborate an early study showing that blinking and sneezing evoked by ocular or intranasal nicotine decreased with repeated application and could be prevented by nicotinic antagonists or by capsaicin pretreatment (Jansco et al. 1961).
Using an in vitro corneal preparation, a population of C-fiber chemonociceptive afferents has been identified that is unresponsive to mechanical and thermal stimuli but responds to noxious chemicals (MacIver and Tanelian 1993a; Tanelian 1991). Such fibers responded to acetylcholine and cholinergic agonists including nicotine over a 10^{-2}-to-10^{-5}-M concentration range, and responses were blocked by nicotinic antagonists d-tubocurare and kappa-bungarotoxin, but not atropine or alpha-bungarotoxin, consistent with mediation via a neuronal nicotinic receptor (MacIver and Tanelian 1993a; Tanelian 1991). In the present study, nicotine (10^{-2}-to-10^{-4}-M concentration range) was highly effective in exciting corneal-conjunctival units in a mecamylamine-antagonizable manner, indicating involvement of a neuronal nicotinic receptor consistent with the possibility that the units received input from chemosensitive corneal afferents.

In addition to salt taste, intraoral application of concentrated (5 M) NaCl evokes irritation that sensitizes with repeated applications (Green 1989) and is cross-desensitized by capsaicin (Gillmore and Green 1993). A large majority of caudalis units responded in a dose-related manner to NaCl. However, responses declined by \sim 22\% over repeated trials (5-min interstimulus interval) in contrast to the increase (sensitization) in human psychophysical irritation ratings.
FIG. 9. (continued)
which were obtained using a much shorter (1-min) interstimulus interval (Green 1989).

Intracutaneous injection of hypertonic solutions is painful (Keele and Armstrong 1964). Various salts including NaCl (0.5–2.5 M) activated a majority of C-fiber nociceptor afferents in the lingual (Wang et al. 1993) and saphenous (Kress and Reeh, 1996) nerves, and lingual responses to NaCl were cross-desensitized by capsaicin (Wang et al. 1993). The tight junction blocker, LaCl3, blocked responses to salts presumably by interfering with access to nerve endings beneath the stratum corneum (Wang et al. 1993). The transduction mechanism for salt irritation is unknown and might speculatively involve depolarization by Na+ influx through amiloride-sensitive channels as in salt taste transduction or an
FIG. 11. Mecamylamine antagonism of unit response to application of nicotine to the cornea-conjunctiva. PSTHs (binwidth: 1 s) show, from left to right, responses to application of nicotine (1%) to the cornea-conjunctiva, the response when nicotine was applied 30 s after corneal application of mecamylamine (0.1%), and the response to nicotine 5 min later.

A substantial fraction of corneal nociceptors responded to NaCl as well as acid and capsaicin given successively (Belmonte et al. 1991; Chen et al. 1997; Gallar et al. 1993). Corneal nociceptor responses to NaCl were of slower onset compared with acid, peaking within 4–6 s and declining slowly during the next 20–30 s (Belmonte et al. 1991; Gallar et al. 1993). By comparison, NaCl-evoked responses of the present corneal-conjunctival units peaked even more slowly (20–40 s) and declined gradually (Fig. 5).

ETHANOL. Ethanol evokes a concentration-dependent irritation on the human tongue (Green 1988), activates cold-sensitive lingual nerve fibers in cats (Hellekant 1965), and increases lingual whole-nerve activity in dogs (Sostman and Simon 1991). Ethanol was presently a highly effective excitant of tongue and corneal-conjunctival units. It is unknown if this action of ethanol might be mediated via activation of polymodal nociceptors, and if so, if stimulus transduction involves a general effect on the terminal membrane. The excitatory effect of ethanol on trigeminal units is probably not due exclusively to activation of cold receptors by evaporative cooling but because ethanol excited some units that were not activated by physical cooling of the tongue or cornea-conjunctiva.

SEROTONIN. 5-HT in human skin elicits pain (Keele and Armstrong 1964) and itch (Hägermark 1992). 5-HT excites a proportion of cutaneous and muscular nociceptors, apparently via the 5-HT–3 receptor subtype (reviewed in Kress and Reeh 1996). 5-HT excited a somewhat lower fraction of caudalis tongue (61%) and corneal-conjunctival units (25%) compared with other chemicals, possibly because the highest concentration used (3%) was only mildly irritating.

ACID AND CO2. In humans, citric acid evokes a dose-related oral irritation (and sour taste); capsaicin cross-desensitized the oral irritation (but not sour taste) (Gilmore and Green 1993, but see also Green 1996) as well as nasal irritation (Geppetti et al. 1993) evoked by citric acid. Lingual nerve afferents were activated by organic acids (Bryant and Moore 1995), and their responses as well as responses of corneal nociceptors to CO2 (Chen et al. 1997) were reduced after capsaicin. Acidification of skin also evokes pain in humans (Steen and Reeh 1993b) that is reduced by topical acetylsaliclycic acid (Schmelz and Kress 1996; Steen et al. 1996), and excites cutaneous nociceptors (Steen et al. 1992, 1995). Our present data are consistent with these findings because acidified buffer solutions evoked responses in caudalis tongue and corneal-conjunctival units in a pH-related manner, and acid-evoked responses of both tongue units tested were abolished after capsaicin.

Polymodal nociceptors with C- or A-delta afferent fibers supplying the cat’s cornea have been shown to respond to acids as well as NaCl, capsaicin (Belmonte et al. 1991; Chen et al. 1997; Gallar et al. 1993; Pozo et al. 1992) and CO2 (Chen et al. 1995, 1997). Corneal nociceptor responses to acetic acid increased with decreasing pH (7–4.5) and did not exhibit tachyphylaxis on repeated application at 2.5-min interstimulus intervals (Belmonte et al. 1991). This is partly consistent with the present data because responses of many corneal-conjunctival units increased with decreasing pH (Fig. 9K); however, successive unit responses decreased significantly across repeated trials (Fig. 9M). Corneal nociceptor responses to acetic acid (60–µl topical instillation) (Belmonte et al. 1991; Gallar et al. 1993) peaked within seconds as did those of the present corneal-conjunctival units but declined more rapidly during a 30-s period compared with the caudalis units the firing of which persisted for >60 s (Fig. 5).

In the present study, 10 of the 14 caudalis tongue units that responded to acid also responded to capsaicin (Table 1). It has been suggested that protons and capsaicin may have a similar mechanism of action at the “capsaicin” (vanilloid) receptor because many small dorsal root ganglion neurons are sensitive to lowering extracellular pH and also to capsaicin (Bevan and Geppetti 1994), and the capsaicin antagonist, capsazepine, blocks responses of tracheal C-fiber nociceptors to both acid (pH 5) and capsaicin (Fox et al. 1995). However, a very recent study of cloned vanilloid receptors has shown that inward depolarizing currents were evoked by capsaicin but not acidification alone and that acidification enhanced the capsaicin-evoked depolarization (Caterina et al. 1997), indicating that capsaicin and protons excite nociceptors via separate mechanisms. This is supported by other studies showing that only a fraction of CO2-sensitive corneal nociceptors also were excited by capsaicin, and responses to capsaicin but not CO2 were blocked by capsazepine (Chen et al. 1997). Furthermore, only 40% of
cultured trigeminal neurons that exhibited increased intracellular Ca\(^{2+}\) levels in response to acid (pH 5.5) also responded similarly to capsaicin (Garcia-Hirschfeld et al. 1995).

CO\(_2\) in solution evokes an oral irritant sensation in humans (Green 1992b; Yau and McDaniel 1990, 1991). CO\(_2\) gas delivered to the cornea (Chen et al. 1995) or nasal mucosa (Anton et al. 1992a; Cain and Murphy 1980) is painful at concentrations >50% and excites corneal nociceptors (Chen et al. 1995, 1997) and trigeminal caudalis neurons (Peppel and Anton 1993). Superfusion with a saturated solution of CO\(_2\) excites polymodal nociceptors in the skin (Steen et al. 1992) and lingual nerve fibers innervating the tongue (Komai and Bryant 1993) in a manner that is blocked by the carbonic anhydrase inhibitor, acetazolamide. Fibers in the chorda tympani also have been reported to respond to CO\(_2\) and carbonated water (Kawamura and Adachi 1967). Interestingly, a substantial fraction of lingual afferents responded to CO\(_2\) but not acid at even lower pH (Komai and Bryant 1993), suggesting that the effect of CO\(_2\) is not solely dependent on tissue pH. Our data are consistent with this because caudalis tongue units responded to carbonated water but not acidified buffer at pH 6 (Fig. 8A). These data suggest that transduction mechanisms for acids and CO\(_2\) may differ, the latter possibly involving a carbonic anhydrase-dependent intracellular acidification. Mechanical stimulation of the tongue by bursting CO\(_2\) bubbles also might contribute to the activation of lingual receptors.

CAPSAICIN. Intraoral capsaicin evokes a burning sensation that increases with repeated application (sensitization) and is smaller or absent when capsaicin is reapplied after a rest period (desensitization) (Dessirier et al. 1997; Green 1989; Karrer and Bartoshuk 1991). Capsaicin is thought to bind a specific capsaicin (vanilloid) receptor (Caterina et al. 1997; Szallasi 1994; Szallasi and Blumberg 1990; Szallasi et al. 1994) to open cation channels (Wood et al. 1988) to depolarize the peripheral terminals of nociceptors (Belmonte et al. 1991; Foster and Ramage 1981; Gallar et al. 1993; Holzer 1991; Szallasi 1994). Capsaicin evokes inward currents in dorsal root ganglion cells in a manner that is antagonized by putative capsaicin antagonists capsazepine and ruthenium red (Bevan and Szolcsányi 1990; Liu and Simon 1994, 1996b,c). Furthermore, topicaly applied acetylsalicylic acid reduced the burning sensation of capsaicin on the skin (Schmelz and Kress 1996), suggesting that nonsteroidal anti-inflammatory drugs (NSAIDs) might antagonize the effect of capsaicin receptor activation. It is interesting that 5–10 min after application of capsaicin to the tongue or eye, there was a significant increase in spontaneous firing of caudalis units that waxed and waned over time (Fig. 7A) consistent with capsaicin’s well-known prolonged irritant sensation as well as the variation in sensory magnitude that is commonly reported. Similarly, after corneal application of capsaicin, there was persistent irregular ongoing activity in nociceptors (Belmonte et al. 1991; Chen et al. 1997; Gallar et al. 1993).

After an initial suprathreshold dose of capsaicin, subsequent responses of caudalis tongue and corneal-conjunctival units to capsaicin were weak or absent (Figs. 2, 3, 5M, and 9O) suggestive of desensitization. This is consistent with reports that human irritant sensations (e.g., Green 1989), inward currents in dorsal root or trigeminal ganglion neurons (e.g., Chard et al. 1995; Cholewinski et al. 1993; Liu and Simon 1996b), and nociceptive behavioral responses of animals (Jansco et al. 1961) elicited by capsaicin are reduced when capsaicin is subsequently reapplied. Furthermore, subsequent application of other irritant chemicals often failed to elicit unit responses, suggestive of cross-desensitization. Cross-desensitization by capsaicin of irritation evoked by other chemicals such as nicotine has been shown psychophysically (Dessirier et al. 1997; Green 1991), and capsaicin desensitized responses of corneal nociceptors to subsequent application of acid or NaCl (Belmonte et al. 1991; Chen et al. 1997; Gallar et al. 1993). The mechanism of capsaicin desensitization is not completely known but is thought to require Ca\(^{2+}\) influx through cation channels opened by the capsaicin receptor. Interestingly, when capsaicin is applied recurrently to the human tongue, which had been previously desensitized by capsaicin, irritant sensations increase across trials suggesting that the excitatory effect of capsaicin can overcome desensitization (Green 1996).

PIPERINE. Piperine, the active chemical in black pepper, produces a burning sensation in the oral cavity that might be discriminable from that of capsaicin (Lawless and Stevens 1988) (see further text). A recent psychophysical study has shown that irritant sensations elicited by capsaicin and piperine exhibit a reciprocal cross-desensitization (Green 1996). Piperine and zingerone (the irritant chemical in ginger) both evoked inward depolarizing currents in trigeminal ganglion neurons that also were depolarized by capsaicin (Liu and Simon 1996c). Depolarizations evoked by piperine (and zingerone) were prevented by the capsaicin antagonist, capsazepine, indicating that piperine may act via the same receptor mechanism as capsaicin (Liu and Simon 1996c) and thus arguing against discriminability between capsaicin and piperine.

MUSTARD OIL. Mustard oil (active chemical, allyl-isothiocyanate) produces a burning sensation on the skin and excites virtually all cutaneous polymodal nociceptors (e.g., Handwerker et al. 1991). Mustard oil vapor delivered to the rat’s nasal sinus or cornea evoked neuronal responses (Meng et al. 1997; Peppe and Anton 1993) and c-fos expression (Anton et al. 1992b; Meng and Bereiter 1996) in trigeminal caudalis. Mustard oil excited one-half of the presently tested caudalis tongue and corneal-conjunctival units (Tables 1 and 2). However, mustard oil was almost always the last chemical to be tested, and the lower incidence of activation might be due to a possible cross-desensitization effect of prior capsaicin. Repeated application of mustard oil to the tongue or eye usually resulted in progressively decreasing unit responses (Figs. 6R and 9Q) although the overall decrease was not statistically significant; in other studies mustard oil has had a sensitizing effect on trigeminal (Hu et al. 1992; Yu et al. 1993) or spinal neurons (Woolf and King 1990).

Coding and discrimination of oral and ocular irritant sensations

In the present study, a substantial fraction of caudalis units responded to a variety of irritant chemicals applied to oral
or ocular mucosa. If such neurons constitute part of a neural circuit mediating the sensation(s) of irritation, then the present data support the idea of a ‘‘common chemical sense’’ because any of a variety of different irritants evokes a similar discharge in a population of trigeminal neurons.

Based on the locations and properties of the present units, it is reasonable to argue that they contribute as second- or higher-order neurons in a pathway mediating irritation. The locations of the present tongue units correspond well with previous reports of lingual afferent terminations (e.g., Shigenaga et al. 1986a) as well as with the distribution c-fos-immunopositive neurons after irritant chemical (Carstens et al. 1995) or mechanical (Strassman and Vos 1993) stimulation of the rat’s tongue. Although we did not presently measure neuronal response latencies to electrical stimulation, the recording loci and fairly short-latency (<5 s; Fig. 5) responses to chemical stimuli are consistent with the possibility that these tongue units may receive input directly from lingual nerve afferents. The locations of many of the present corneal–conjunctival units (Fig. 1) were at the rostral end of the caudal medullary–upper cervical spinal terminal regions of corneal afferents, while some were intermediate between this and the second corneal termination zone at the caudalis-interpolaris transitional area (Meng and Bereiter 1996; Shigenaga et al. 1986a; Strassman and Vos 1993). Again, the fairly rapid onset of chemically evoked responses of these units (Fig. 6) are consistent with the possibility that these represent second-order neurons although it is certainly possible that some were higher order.

A caveat of the present study is that we did not test for rostral projections of the recorded units and therefore are not certain if they contributed to ascending sensory pathways or were local interneurons. Many other studies have shown that WDR and nociceptive-specific units similar to those recorded presently have ascending projections to contralateral thalamus or parabrachial area (e.g., Meng et al. 1997). Considerable indirect evidence indicates that WDR neurons play an important role in signaling sensory-discriminative aspects of pain (Dubner et al. 1989; Mayer et al. 1975). Therefore it is not unreasonable to expect that some of the present neurons may contribute to ascending pathways signaling chemical irritation.

Although the presently recorded tongue and corneal–conjunctival units in caudalis showed characteristics consistent with a role in signaling orofacial chemical irritation and pain, it is unlikely that these are the only neuronal populations involved. As noted in the INTRODUCTION, neurons in subnuclei interpolaris and oralis also respond to noxious orofacial stimuli and may contribute to ascending sensory pathways mediating pain. Furthermore, neurons in the nucleus of the solitary tract (NTS) also might play a role in signaling orofacial pain and irritation. Numerous anatomic studies have demonstrated trigeminal afferent (primarily mandibular) projections to gustatory and nongustatory areas of the NTS (Beckstead and Norgren 1979; Hamilton and Norgren 1984; Jacquin et al. 1983; Marfurt and Rajchert 1991; Pfläger and Arvidsson 1988; Takemura et al. 1987, 1991; Torvik 1956). Electrophysiological studies have shown that many neurons throughout rostral NTS respond to intraoral mechanical or thermal stimuli or to both mechanical and gustatory stimuli (Halsell et al. 1993; Hayama et al. 1985; Ogawa and Hayama 1984; Ogawa et al. 1984, 1988; Sweazey and Bradley 1989; Travers and Norgren 1995). A recent study demonstrated significant increases in c-fos–immunoreactive neurons in NTS, as well as in superficial layers of dorsomedial caudalis, after application of certain irritant chemicals to the rat’s tongue (Carstens et al. 1995). It is also conceivable that intraoral irritation could be signaled via convergence of secondary trigeminal caudalis projections onto gustatory relay neurons in the parabrachial nucleus (Hayama and Ogawa 1987; Ogawa et al. 1982). However, it is currently unknown if neurons in NTS or parabrachial areas receiving convergent somatosensory and gustatory inputs respond to irritant chemicals.

Given the broadly tuned responsiveness of many caudalis neurons to different irritant chemicals, it is interesting to consider whether humans can discriminate qualitative differences in chemically evoked irritant sensations or if there is one common chemesthetic sense. Discrimination between oral irritant sensations elicited by acids or salts is likely aided by concomitant gustatory (sour or salty) sensations. Orally administered capsaicin and piperine, which are tasteless, are reported to elicit qualitatively different sensations of stinging, biting, and piercing versus itching (Lawless and Stevens 1990). Histamine on the tongue evokes a ‘‘pungent burning’’ or horseradish-like sensation (Heubner 1925) or pricking sensation (Carstens, unpublished observation) that may be subjectively distinct from the burning sensation elicited by capsaicin. However, more studies are needed to determine if humans can discriminate among chemically evoked oral irritant sensations.

The ability to qualitatively discriminate different corneal stimuli has been debated. Mechanical and thermal (warming and cooling) stimuli delivered to the human cornea or conjunctiva have been reported to evoke sensations of irritation at low intensities and pain at higher intensities (Beuerman and Tanelian 1979; Keshali 1960; Shirmer 1963) with the conjunctiva being less sensitive than the cornea (Norn 1973). There are few studies of corneal sensations elicited by noxious chemical stimuli. A variety of airborne irritant chemicals elicited irritation at threshold concentrations that varied as a function of carbon chain length; the threshold for irritation elicited by a given chemical was similar when delivered to eye or nasal sinus and was higher than the threshold for odor detection (Cometto-Muniz and Cain 1995). It was reported anecdotally that acetylcholine elicits a burning or pricking pain sensation on the human cornea (MacIver and Tanelian 1993b). Capsaicin in the eye elicits sharp pain (Dupuy et al. 1988) and CO₂ delivered to the human cornea also elicits sensations of pricking, stinging, or irritation (Chen et al. 1995; Gonzalez et al. 1993). However, there are reports that humans can correctly identify cool and low-threshold mechanical stimuli delivered to the cornea (Acosta et al. 1996; Lele and Weddell 1956), whereas both hot and chemical (50% CO₂) stimuli are judged to be irritating (Acosta et al. 1996). Histamine instilled into the eye also produces sensations of irritation at low doses and burning or sticking pain at higher doses in humans (Heubner 1925; Keele and Armstrong 1964). Histamine (100–1,000 μg in 20 μl) (Woodward et al. 1995) or prostaglandins (Woodward et al. 1996) instilled into the albino guinea pig eye elicited episodes of hindlimb
scratching directed toward the eye, suggestive of itch. It is presently uncertain how distinct sensations of ocular itch versus pain are mediated, given the present data showing that many units respond to both histamine, a pruritic chemical, as well as capsaicin and other irritants that evoke pain. More studies are needed to establish if there are subpopulations of chemospecific trigeminal neurons that might be able to selectively signal ocular itch versus pain (see Carstens 1997 for further discussion).

The present data, showing that many units responded to mechanical stimuli, noxious heat, irritant chemicals, and sometimes also cooling of the cornea-conjunctive, can be taken as evidence favoring a unitary sensation of corneal irritation and pain. It is conceivable that further psychophysical studies will reveal a greater degree of discriminability of corneal sensory qualities, which might be signaled by other trigeminal neurons not presently encountered that show a greater degree of selectivity in chemical responsiveness. Discrimination might be coded by preferential responses of individual trigeminal neurons to one chemical while responding at a lower frequency to other irritant chemicals. The nervous system would discriminate by comparing inputs from a population of such preferentially responsive neurons. If future studies reveal the existence of more chemically selective trigeminal neurons, discrimination might be accomplished using both a specificity code and across-fiber comparison as postulated for taste sensation. Many neurons at different levels in the gustatory pathway respond to a variety of tastant stimuli, and methods such as cluster analysis have been used in an attempt to identify groups sensitive to particular tastants (Scott and Plata-Salaman 1991). Such methods may eventually be applied to chemesthesia, although it first will be important to develop a panel of irritant chemicals that are matched in terms of sensory intensity.

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