Sensitization, Desensitization and Stimulus-Induced Recovery of Trigeminal Neuronal Responses to Oral Capsaicin and Nicotine

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INTRODUCTION

Capsaicin, the pungent chemical in red chili pepper, induces a burning/pricking sensation in humans and nocifensive behavior in animals when applied to the oral or ocular mucosa and skin (see Buck and Burks 1986; Holzer 1988, 1991; Szallasi and Blumberg 1999; Szolcsányi 1990 for reviews). This sensation is believed to be mediated by the activation of a capsaicin (vanilloid) receptor, a ligand- and heat-gated ion channel present in subpopulations of small-diameter dorsal root ganglion neurons including nociceptors, one subtype of which (VR-1) has been recently cloned (Caterina et al. 1997; Tominaga et al. 1998). Indeed, “knockout” mice lacking the VR-1 receptor exhibit a drastic reduction in nocifensive behavioral responses to capsaicin or noxious heat (Caterina et al. 2000). In human studies, capsaicin-evoked irritation usually occurs in intensity across trials of repeated application at a short interstimulus interval (ISI), a phenomenon termed “sensitization” (Dessirier et al. 1997; Green 1989). Sensitization has been observed with intra-oral and cutaneous application of capsaicin (Green 1998). The cellular mechanisms underlying sensitization may involve spatial recruitment of VR-1 receptors in nociceptor endings, an increase in excitability of nociceptors peripherally via, for example, local release of inflammatory mediators, or an increased excitability of central neurons in the nociceptive pathway (see DISCUSSION). After the initial application of capsaicin followed by a rest period, reaplication of capsaicin results in a markedly reduced sensation, a phenomenon commonly referred to as “desensitization” (Dessirier et al. 1997; Green 1989, 1991; Karrer and Bartoshuk 1991), a term coined by Jancsó and Jancsó (1949; see Szolcsányi 1990 for reviews). This sensitization is believed to be mediated by the activation of a capsaicin-induced receptor exhibit a drastic reduction in nocifensive behavioral responses to capsaicin or noxious heat (Caterina et al. 2000).

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Presumed capsaicin-sensitive nociceptors in the oral cavity project via the lingual branch of the trigeminal nerve to terminate in the brain stem trigeminal complex (Amano et al. 1986; Carstens et al. 1995, 2000; Kruger and Michel 1962; Strassman and Vos 1993; Yokota 1975), where they activate neurons in subnuclei dorsomedial trigeminal subnucleus caudalis (Vc) (Carstens et al. 1998; Simons et al. 1999) and oralis (Dallel et al. 1999). Individual units in the dorsomedial aspect of Vc respond to application of capsaicin to the tongue in a concentration-dependent manner (Carstens et al. 1998). Responses of Vc (Carstens et al. 1998) and spinal dorsal horn (Carstens 1997) neurons to repeated application of capsaicin at a long (5-min) ISI exhibited tachyphylaxis. However, it is not known whether such neurons exhibit a progressive increase in firing with application of capsaicin at a shorter ISI. One aim of the present study was to investigate this question in nociceptive Vc units that respond to irritant chemical stimulation of the tongue. We hypothesize that Vc neurons mediate sensations of oral irritation, and that their response patterns to repeated application of capsaicin should reflect changes in sensation as evaluated psychophysically.

Recurrent application of capsaicin to previously desensitized epithelium induces irritation that again rises to approach the intensity achieved prior to desensitization (Green 1996; Green and Rentmeister-Bryant 1998), a phenomenon called “stimulus induced recovery” (SIR). It has been proposed that desensitization and SIR are mediated by opposing cellular processes, such that recurrent application of capsaicin induces excitation of nociceptors that overcomes desensitization (Green 1993; Green and Rentmeister-Bryant 1998). Alternatively, SIR might reflect the recruitment of low-affinity capsaicin receptors that were not desensitized during the initial application of capsaicin (Green and Rentmeister-Bryant 1998). A second aim of this study was to determine whether Vc neurons exhibit a recovery in responsiveness to capsaicin, similar to SIR, following an initial period of capsaicin application that results in desensitization.

In contrast to capsaicin, repeated application of nicotine to the tongue elicits an irritant sensation, the magnitude of which significantly decreases across trials (Dessirier et al. 1997, 1999a). Nicotine is a well-known irritant of ocular, nasal and oral mucosae, and skin (Cain 1980; Grieff et al. 1993; Hummel et al. 1992; Janasco et al. 1961; Jarvis and Assil 1988; Keele and Armstrong 1964), presumably via binding to neuronal nicotinic acetylcholine receptors (nAChRs) (see Holladay et al. 1997; Lindstrom et al. 1996 for recent reviews) expressed in the epithelial endings of nociceptors (Stein and Reeh 1993; Tanelian 1991). Tachyphylaxis is also observed in Vc (Carstens et al. 1998) and spinal dorsal horn (Jinks and Carstens 1999) neuronal responses to repeated application of nicotine at relatively long (5–10 min) ISI. The mechanism presumably involves desensitization of nAChRs and/or Ca2+-mediated changes in nociceptor excitability as mentioned above for capsaicin. A third aim of this study was to determine whether repeated or continuous application of nicotine elicited a pattern of Vc neuronal firing characterized by a progressive decline similar to that observed in the psychophysical studies.

In the present experiments we sought nociceptive Vc neurons that responded to irritant chemicals delivered to the tongue. A previous study (Carstens et al. 1998) showed that nociceptive-specific (NS) and wide dynamic range (WDR)-type Vc units frequently responded to application of a variety of irritant chemicals, including acidic stimuli (pH 1–3). Repeated application of acid did not lead to tachyphylaxis in successive Vc unit responses. Because of this nondenosing property, we reasoned that acid might provide a good diagnostic test of the responsiveness of Vc neurons to additional chemicals such as capsaicin and nicotine. In the present study we used pentanoic acid, which excites lingual nerve afferents (Bryant and Moore 1995) and is irritating when applied to the human oral mucosa (Simons et al. 1999). The rationale for using pentanoic acid stems from our earlier study (Simons et al. 1999) that investigated oral irritation elicited by carbonated water; pentanoic acid was used for comparison because it has a lipid solubility similar to that of CO2. A fourth aim of the present study was therefore to verify that pentanoic acid excites capsaicin- and nicotine-sensitive Vc units in a manner that does not cause tachyphylaxis, and thus might serve as a diagnostic tool in identifying chemonociceptive Vc neurons. An abstract of portions of this work has appeared (Dessirier et al. 1999b).

**Methods**

**Animals**

Twenty-nine adult male Sprague-Dawley rats (Simonsen, Gilroy, CA; 410–540 g) were used. To obviate any possible effects of circadian rhythms (Loetsch et al. 1998), the experiments were always started between 12 and 2 p.m. All protocols were approved by the UC Davis Animal Use and Care Advisory committee.

**Recording and stimulation methods**

The methods were similar to those employed previously (Carstens et al. 1998; Simons et al. 1999) and are summarized here. Rats were anesthetized with thiopental (induction: 80 mg/kg ip; maintenance: 10 mg · kg·h−1 · h−1 iv). The caudal medulla was exposed by laminectomy at C1 and removal of the caudal portion of the occipital bone. The medulla/upper cervical spinal cord was suspended by placing the head in a stereotaxic frame in a ventroflexed position, and placing a vertebral clamp at C1. The dura mater was removed, and an agar pool was formed over the brain stem. A small clip was placed over the upper and lower incisors in such a way as to keep the mouth open and the tongue easily accessible. Isotonic saline was applied frequently to the tongue to prevent desiccation.

A tungsten microelectrode (F. Haer, 10.120.33.4 on October 26, 2016 http://jn.physiology.org/ Downloaded from) was advanced into the superficial medulla to record extracellular single-unit activity, which was amplified and displayed by conventional means and fed to a computer for storage and analysis (Forster and Handwerker 1990). Recordings were restricted to superficial layers (50–500 μm below the surface) of the dorsomedial aspect of Vc. Only units with a mechanosensitive receptive field that included the ipsilateral dorsal surface of the tongue, and that additionally responded to noxious heat (0.1 ml of deionized water; ~54°C) and 1 M pentanoic acid applied there, were studied further. The rationale for using pentanoic acid was that it is lipid soluble like capsaicin, and in pilot studies Vc units that responded to pentanoic acid also responded to subsequent application of capsaicin and/or nicotine, as confirmed presently. We were not able to use capsaicin to characterize units because of its desensitizing effect, and only used capsaicin during formal testing on one unit per rat.

The chemical stimuli employed were pentanoic acid 1 M, nicotine 10% (616 mM; pH 10) and capsaicin 330 μM (100 ppm; diluted from a 0.1% stock solution of capsaicin in 95% ethanol); all were purchased from Sigma Chemical (St. Louis, MO). This capsaicin concentration was used because in pilot experiments none of eight units tested...
responded to lower (5 or 10 ppm) concentrations of capsaicin, consistent with an earlier study showing excitation of Vc units by 100 ppm, but not 10 ppm, capsaicin (Carstens et al. 1998). Chemicals were applied under six different conditions: 1) repeated discrete application of pentanoic acid at 5-min ISI, 2) single application of capsaicin, 3) repeated discrete application of capsaicin at 1-min ISI, 4) repeated discrete application of nicotine at 1-min ISI, 5) constant-flow application of capsaicin, or 6) constant-flow application of nicotine.

In approximately one-half of the experiments, pentanoic acid (0.1 ml) was applied three or more times in succession at a 5-min ISI via syringe. This was done before any other chemicals were tested. The fluid was applied manually at room temperature to form a bolus on the anterior dorsal surface of the tongue, and was rinsed off with approximately 1 ml of isotonic saline after 60 s.

In some experiments, the next stimulus tested was nicotine. It was applied either repeatedly at 1-min ISI, or by continuous flow, in separate experiments. For repeated application, 0.1-ml volumes of nicotine were applied as described above for a total of 15 repeated applications, followed by rinsing the tongue with approximately 2 ml of isotonic saline. For continuous flow, the nicotine was delivered at a constant rate of 0.32 ml/min onto the dorsal surface of the tongue bilaterally from a PE 60 tube connected to a syringe that was driven by an electronically controlled pump (Sage Instruments; Div. Orion Research, Cambridge, MA). The nicotine was flowed for the same 15-min period, after which the tongue was rinsed with saline. A high (10%) concentration of nicotine was chosen because it elicited responses that were of approximately the same magnitude as those elicited by 330 μM capsaicin.

Because the rat’s head was in a ventroflexed position, the applied fluid bolus was visually confirmed to be restricted to the dorsal lingual surface and did not contact other oral tissue if it dripped off. When chemicals were applied by constant flow, the outflow port of the tubing was placed more posteriorly on the tongue so that fluid flowed toward the tip of the tongue. A fluid bolus built up and dripped off of the tip of the tongue approximately every 10 s, and by visual inspection was confirmed not to contact any other tissue.

In some experiments, capsaicin was tested following characterization with pentanoic acid. The pentanoic acid did not induce tachyphylaxis (see RESULTS). In other experiments, capsaicin was tested >60 min after first testing with nicotine. In pilot studies and previous experiments (Carstens et al. 1998) we have found that any tachyphylaxis induced by nicotine had recovered by this time. Moreover, we have no evidence for cross-tachyphylaxis from nicotine to capsaicin (Carstens et al. 1998; Dessirier et al. 1997). The capsaicin was applied either 1) once in a 0.1-ml bolus that was left on for 25 min, after which the tongue was rinsed with saline, 2) repeatedly at 1-min ISI for a total of 25 applications, followed by a saline rinse, or 3) by constant flow as described for nicotine, except that the period of flow was 25 min followed by a saline rinse. The 25-min duration was selected for capsaicin to ensure that a stable firing level had been achieved following sensitization. The shorter (15-min) nicotine application period was selected based on our observation that unit responses peaked within the first 3–4 min of nicotine application and then adapted to the prenicotinic level well before 15 min.

Following the 25-min period of capsaicin application, a 30- to 60-min rest period was imposed. Capsaicin was then reapplied in exactly the same manner as before (i.e., single bolus, 25 discrete applications, or continuously) to test for SIR.

At the conclusion of the recording session, an electrolytic lesion was made, the rat was killed by overdose with thiopental, and the brain stem was removed and postfixed in 10% Formalin. At least 1 wk later the brain stem was cut in 50-μm frozen sections that were counterstained with Neutral Red for microscopic identification of lesion sites (Fig. 1).

**RESULTS**

**Unit characterization**

Twenty-nine units responded to noxious pinch, noxious heat (54°C water) and pentanoic acid 1 M. Of these, 17 also responded to nonnoxious tactile stimuli and were thus categorized as WDR. The 12 remaining units were categorized as NS. The mechanical receptive field was usually limited to the ipsilateral tongue and tip bilaterally, but in six units also included portions of the ipsilateral lower lip and chin. Spontaneous activity in these units was generally low and seldom exceeded 5 Hz. Figure 2 shows a typical example of an NS unit that responded briefly to heat and pinch of the tongue, and with a more prolonged discharge to application of pentanoic acid (left-to-right PSTHs). Recording sites were in and just medial to the superficial laminae of dorsomedial Vc (Fig. 1), as in our earlier studies (Carstens et al. 1998; Simons et al. 1999). There were no apparent differences in the responses of WDR and NS units to the various chemicals tested, so data from both unit classes have been pooled.

**Responses to pentanoic acid**

Pentanoic acid was used as a nondesensitizing agent to test unit responsiveness to irritant chemicals. These experiments were therefore undertaken to verify that repeated application did not induce sensitization or tachyphylaxis. Figure 3A shows...
averaged PSTHs of the responses of 14 units to 3 sequential applications of pentanoic acid at 5-min ISI. Figure 3B plots individual (thin lines) and mean (thick line) unit responses to the three applications of pentanoic acid (not corrected for spontaneous activity). Very few units exhibited tachyphylaxis or sensitization in successive responses (Fig. 3B), and when averaged over 60 s the mean responses did not significantly vary across trials. However, the mean response reached a maximal rate more quickly in the first trial compared with trials 2 and 3. Analysis of the initial 10-s period following stimulus onset (Fig. 3A, light bars in PSTHs) revealed a significant trial effect (ANOVA, $P = 0.044$), with the initial 10-s response component being significantly larger on the first compared with later trials.

Sensitization and SIR with repeated application of capsaicin

Figure 4A shows an individual Vc unit’s response to 25 repeated applications of capsaicin (arrows, 1-min ISI). The response increased over the initial six to eight trials, consistent with sensitization, and then achieved a plateau with some waxing and waning. Figure 4B shows the same unit’s responses to the identical series of capsaicin stimuli recorded after a 60-min rest period. Initially, the spontaneous firing level was similar to the precapsaicin level. Repeated application of capsaicin eventually induced an increase in firing, but after a considerable delay compared with the initial series. Furthermore, the maximal firing rate during the second series never reached that achieved in the initial series. This is consistent with the interpretation that desensitization was overcome to result in a partial SIR.

These patterns of sensitization and partial SIR were consistent across all 11 units studied. The averaged PSTH of the 11 units is shown in Fig. 5A, where it is apparent that the firing increased across the initial eight applications to reach a plateau. Figure 5B replots the PSTH with a larger (60-s) binwidth. The mean firing rate increased significantly by the second application ($P = 0.013$), and increased further to reach a plateau by the seventh application ($P = 0.045$). Figure 5C shows the averaged PSTH to the second series delivered after a 30- to 60-min rest period, revealing several points. First, the mean spontaneous level prior to the first capsaicin stimulus was not significantly different from the precapsaicin level (Fig. 5A). Second, the response increased more slowly compared with the initial series and was not significantly different from spontaneous levels until the 8th application (Fig. 5D; $P = 0.013$), reaching a plateau around the 10th application. This pattern is consistent with a recovery from desensitization followed by a partial SIR. Finally, the maximal mean firing rate was significantly lower compared with that attained in the initial series ($P = 0.001$).
Sensitization and SIR with constant-flow application of capsaicin

Figure 6A shows an example of a Vc unit’s response to application of capsaicin by constant flow. The firing rate increased over the initial 8 min to achieve a plateau with waxing and waning, much like repeated application of capsaicin. Figure 6B shows the same unit’s response to reapplication of capsaicin by constant flow after a 60-min rest period. There was considerable a delay before the firing rate increased to nearly the same level as in the initial trial. This pattern was consistent across the seven units tested.

The averaged PSTH for these seven units is shown in Fig. 7A, again revealing a rapid increase in firing during the first few minutes, consistent with sensitization. Figure 7B shows the...
averaged PSTH with 60-s bins. Neuronal firing increased significantly by the third minute of application \( (P = 0.017) \), and continued to increase significantly \( (P = 0.03) \) until \( 9.6 \pm 1.2 \) (SD) min before reaching a plateau. Figure 7C shows the averaged PSTH to reapplication of capsaicin by constant flow following a 60 min rest period. Prior to the onset of the stimulus, the spontaneous firing level was not significantly different from the precapsaicin level. During capsaicin application, there was a marked delay before the firing rate began to increase. This is also evident in the expanded PSTH of Fig. 7D: the increase in firing rate was only significant by the 12th min.

In this case, the SIR appeared to be nearly complete since the maximal firing rate was not significantly lower than that of the first trial \( (P = 0.2) \).

### Single application of capsaicin

To verify that repetitive or continuous application of capsaicin is necessary for sensitization, we also tested the effect of a single application of capsaicin. The capsaicin was placed on the tongue as a bolus while recording for 25 min. The example in Fig. 8A shows that single-trial application of capsaicin...
induced a modest increase in firing. After a 60-min rest period, the capsaicin was reapplied (Fig. 8B), and after a delay induced a small increase in firing. Figure 9A shows the averaged PSTH for eight Vc units tested with a single application of capsaicin. The firing increased shortly after application and continued to increase slightly over the next several minutes to reach a plateau with waxing and waning. Figure 9B shows that the firing had increased significantly by the second minute after capsaicin application ($P < 0.035$) and continued to slowly increase until the eighth minute. The mean maximal firing rate was significantly lower compared with the sequential stimulation condition ($P = 0.05$).

When capsaicin was reapplied after the rest period, the averaged firing remained constant with no significant increase over time (Fig. 9, C and D). This indicates an absence of SIR following a singular reapplication of capsaicin.

**Nicotine**

In contrast to capsaicin, repeated or continuous application of nicotine had a biphasic effect on neuronal firing, with an initial, short-lasting excitation followed by a marked reduction. Figure 10A shows an example of a unit whose firing increased during the first four applications of nicotine, followed by...
reduced firing even though application of nicotine continued. Figure 10B shows a similar effect of constant-flow nicotine, with the unit initially increasing its firing, followed by a decline to spontaneous levels. This biphasic pattern was seen in all units tested. The averaged PSTH of 12 Vc units to repeated discrete application of nicotine is shown in Fig. 11A. When the PSTH binwidth was expanded to 1 min (Fig. 11B), the peak firing rate at the third application was significantly greater than prenicotine ($P = 0.012$), followed by a decrease in neural activity to levels equivalent to spontaneous activity by the 12th application ($P = 0.67$). Constant-flow application of nicotine had an even more pronounced biphasic effect, with an increase in firing over the first 3 min ($P < 0.01$), followed by a reduction in firing to the prenicotine level (Fig. 11, C and D).

DISCUSSION

The results show that WDR and NS-type units in superficial laminae of dorsomedial Vc respond to lingual application of pentanoic acid, nicotine, and capsaicin. The pattern of Vc unit responses to continuous (flow) or repeated discrete application
of capsaicin was different from that elicited by nicotine. Capsaicin elicited a rapid increase in firing that reached a plateau after approximately 8 min, consistent with sensitization. In contrast, nicotine elicited an increase in firing over the initial 3 min, followed by a decline in firing consistent with adaptation or desensitization. These results are discussed in terms of human psychophysical judgements of irritation elicited by the same chemicals, and the possible neural mechanisms involved.

Vc unit response to acid

In the present study, all Vc units that responded to pentanoic acid additionally responded to subsequent nicotine and/or capsaicin, consistent with our prior study showing that Vc units often respond to a wide variety of irritant chemicals (Carstens et al. 1998). Furthermore, Vc unit responses to repeated application of pentanoic acid were usually stable across trials, although a few showed progressive increases or decreases indicative of sensitization and tachyphylaxis, respectively (Fig. 3B). Interestingly, the initial 10-s component of the mean unit response to pentanoic acid was significantly larger on the first trial compared with later trials (Fig. 3A). This might be explained by the development of desensitization following the initial application of acid, which reduced the initial component of subsequent Vc unit responses but which was overcome by the excitatory effect of the acid. A lack of sensitization or tachyphylaxis was also reported for responses of corneal nociceptors to acetic acid (Belmonte et al. 1991) and support our present use of pentanoic acid as a non-desensitizing agent that predicts Vc neuronal sensitivity to other chemical irritants including capsaicin. However, we did not test whether units insensitive to pentanoic acid nonetheless responded to capsaicin, and therefore cannot address the issue of whether some Vc units selectively respond only to certain classes of irritant chemicals (see Carstens et al. 1998).

In a previous study we showed that application of carbonated water to the tongue activated Vc units in rats, and induced an irritant sensation in humans, that were both significantly attenuated by the carbonic anhydrase inhibitor dorzolamide (Simons et al. 1999). Vc unit responses to HCl, and human sensation elicited by pentanoic acid, were not affected by dorzolamide, arguing that distinct transduction mechanisms underlie the excitation of intraoral nociceptors by CO2 compared with dissociated acids. Although we presently did not investigate the mechanism by which pentanoic acid excited Vc units, possibilities are intracellular acidification (Steen et al. 1999), extracellular proton-induced activation of acid-sensitive ion channels (ASICs) (Waldmann et al. 1997; see Waldmann and Lazdunski 1998 for review), or direct activation of VR-1 receptors (Caterina et al. 2000; Tominaga et al. 1998). The latter possibility receives support from the recent observation that dorsal root ganglion neurons and cutaneous nociceptive afferent fibers from “knockout” mice lacking the VR-1 receptor exhibit a marked reduction in the incidence of activation by acidic stimulation compared with wild-type mice (Caterina et al. 2000). Since all of the acid-sensitive Vc units recorded presently also responded to intense (54°C) noxious heat as well as capsaicin and nicotine, it is suggested that they received convergent afferent input from lingual nociceptors expressing VR-1 receptors, nAChRs, possibly ASICs, and possibly also VR-L-1 receptors that are activated by intense heat but not capsaicin or acid (Caterina et al. 1999).

Capsaicin sensitization

Application of capsaicin by constant flow, or by repeated application at 1-min ISI, resulted in a rapid increase in unit firing over the first several minutes, consistent with sensitization observed in human psychophysical studies (Dessirier et al. 1997; Green 1989; Lawless and Gillette 1985). By comparison, a single application of capsaicin resulted in a significant elevation in unit firing (Fig. 9), which, however, grew much more gradually and reached a significantly lower maximal rate consistent with our earlier report (Carstens et al. 1998). We speculate that the waxing and waning character of the ongoing capsaicin-evoked unit responses may be a neural correlate of the variation in sensory intensity that is commonly perceived when eating food spiced with capsaicin.

The simplest explanation for the difference between single and repeated/continuous application is the capsaicin concentration achieved at the level of nociceptor terminals in the lingual epithelium. Thus singular application of capsaicin would lead to an initial increase, followed by a decrease, in intraepithelial concentration dependent on the rates of diffusion and clearance. With repeated/continuous application, the intraepithelial concentration would presumably rise to some maximal level. The overall higher mean firing rate observed with repeated/continuous application of capsaicin might reflect the continued maintenance of a higher intraepithelial concentration. In addition, more capsaicin would be available to diffuse over a larger distance, thereby recruiting more capsaicin-sensitive nociceptive endings. Such spatial summation might also contribute to the sensitization of Vc unit firing (see below).

Sensitization could be mediated via a peripheral effect of capsaicin on nociceptive endings, by central sensitization of Vc units, or both. There are, to our knowledge, no reports of increases in primary afferent or central neuronal firing with repeated application of capsaicin. Instead, responses of trigeminal ganglion neurons (Liu and Simon 1996b,c), corneal nociceptors (Belmonte et al. 1991; Gallar et al. 1993), or spinal and Vc units (Carstens 1997; Carstens et al. 1998) exhibit tachyphylaxis or no change in response to repeated application of capsaicin. However, the ISIs used in these studies were longer (>3 min) than those used to induce sensitization in psychophysical studies (1 min or less). Nonetheless, cultured trigeminal ganglion neurons exposed to 200 nM capsaicin do not seem to exhibit any increased response even at 1 min ISIs (B. Bryant, personal communication), although such in vitro patch-clamp studies do not necessarily reflect the responses of peripheral nerve endings in situ. It is also conceivable that some temporal summation is required to induce an increased firing of capsaicin-sensitive nociceptors, or even activation of “silent” or “sleeping” nociceptors (see Treede et al. 1992); such recruitment would have to occur rapidly enough (i.e., within 3–8 min) for such nociceptors to contribute to sensitization. Another potential mechanism underlying sensitization at short ISI is the capsaicin-evoked peripheral release of substance P from nociceptive endings, leading to buildup of inflammatory mediators that in turn depolarize the nociceptor endings (Holzer 1988).

Alternatively, sensitization may be mediated by a central
enhancement of Vc neuronal responses. Intradermal or orofacial capsaicin elicits pain and initiates the development of primary (thermal and mechanical) hyperalgesia at the site of application, and secondary mechanical hyperalgesia in a larger surrounding area (Ali et al. 1996; Green and Cruz 1998; LaMotte et al. 1991) that was not associated with sensitization of nociceptors in the region of secondary hyperalgesia (Bau mann et al. 1991). Intradermal capsaicin sensitized monkey spinothalamic tract neurons, as indicated by expansion in their cutaneous receptive fields and enhanced responsiveness to peripheral stimuli (Simone et al. 1991). Central sensitization might explain the rise in Vc units’ response to repeated/continual application of capsaicin observed presently, and the concomitant increase in perceived irritation reported in human studies (Dessirier et al. 1997; Green 1989).

Other recent studies have demonstrated central sensitization of Vc neurons. Activation of intracranial dural nociceptors by irritant chemicals led to lowered thresholds and enhanced responses of nociceptive Vc units to both dural and facial cutaneous stimuli (Burstein et al. 1998; Yamamura et al. 1999). Injection of mustard oil into the temporomandibular joint (TMJ) or facial skin resulted in cutaneous receptive field expansion indicative of sensitization, with the effect from TMJ being larger (Hu et al. 1992; Yu et al. 1993). Similarly, inflammation of the TMJ or perioral skin with complete Freund’s adjuvant led to enhanced excitability of nociceptive (and nonnociceptive) units in Vc, with the TMJ inflammation having a stronger effect (Iwata et al. 1999). The initial increase in Vc responses to repeated/continual capsaicin observed presently might reflect central sensitization similar to that elicited by mustard oil or inflammatory agents. A possible mechanism for the central sensitization is “wind-up” in which repetitive stimulation of C-fiber nociceptors elicits progressively greater sensory and central neuronal responses (Arendt-Nielsen et al. 1996; Magerl et al. 1998; Mendell and Wall 1965; Nikolajsen et al. 1996; Price 1972; Price et al. 1977, 1994) via an NMDA receptor-dependent increase in cellular excitability (see Dickenson et al. 1997 for review). We did not presently test whether the sensitization could be reduced or prevented by NMDA receptor antagonists. Clearly, further studies are needed to determine whether the sensitization of Vc units to maintained capsaicin on the tongue is mediated primarily via a peripheral and/or central site of action.

**Capsaicin desensitization and SIR**

When capsaicin was applied as a single bolus, the second application elicited no response, consistent with desensitization (Fig. 9). When capsaicin was applied repetitively or by constant flow, the second application series again elicited a significant increase in firing that, however, was delayed (Figs. 5 and 7) consistent with desensitization followed by SIR.

Vc units continued to fire during prolonged capsaicin stimulation (Figs. 4–7), whereas there was a marked tachyphylaxis in successive responses of Vc units to capsaicin repeated at a long (5-min) ISI with no intervening capsaicin (Carstens et al. 1998). This suggests that the continued presence of capsaicin overcomes its desensitizing effect, consistent with previous psychophysical results (Green 1993).

The capsaicin concentration used in the present study, while high (330 µM), is within the range of concentrations used in human studies (e.g., Green and Rentmeister-Bryant 1998; Karrer and Bartoshuk 1991). Nonetheless, we cannot rule out the possibility that capsaicin had nonspecific effects on nociceptor terminals (Holzer 1988) that contributed to the presently observed pattern of neuronal firing. Interestingly, rats appear capable of detecting the presence of capsaicin in water at much lower concentrations (1 ppm = 3.3 µM; unpublished observations). If chemonociceptive Vc neurons code for irritation, they should exhibit a similarly low threshold. The higher threshold of the present Vc units might reflect the use of barbiturate anesthesia.

Like sensitization, recovery of Vc neuronal firing (“SIR”) appears to require repeated or continuous application of capsaicin to develop (Figs. 4–7) and was not induced by single-trial application (Figs. 8 and 9). SIR might thus involve cellular mechanisms similar or identical to those underlying sensitization. In the present study, the SIR of Vc responses induced by repeated capsaicin application was incomplete (Fig. 5, C and D) in contrast to complete SIR seen in human studies (Green 1996), although with continuous flow SIR was nearly complete (Fig. 7, C and D). Species differences and cognitive or methodological factors (Green 1996; Green and Rentmeister-Bryant 1998, Prescott 1999) might account for the different results. As noted in the Introduction, SIR may result from opposing cellular processes in which excitation of nociceptors overcomes desensitization (Green 1993; Green and Rentmeister-Bryant 1998), or the recruitment of nondesensitized low-affinity capsaicin receptors (Green and Rentmeister-Bryant 1998). Future studies of sensitization, desensitization, and SIR in nociceptors will be important in understanding the mechanism of action of capsaicin as a topical analgesic and potential limiting factors such as SIR.

**Nicotine**

In contrast to capsaicin, repeated or continuous application of nicotine to the tongue elicited an excitatory response only during the first 3 min, followed by a decline in Vc firing (Figs. 10 and 11). This pattern is consistent with adaptation or desensitization to the initial excitatory action of nicotine. We previously showed that responses of Vc (Carstens et al. 1998) and spinal dorsal horn (Jinks and Carstens 1999) neurons to repeated application of nicotine at 5- to 10-min ISIs exhibit significant tachyphylaxis. Nicotine presumably excites peripheral nociceptors via nAChRs (Alimohammadi and Silver 2000; Carstens et al. 1998, 2000; Dessirier et al. 1998; Steen and Reeh 1993; Tanellin 1991) to activate Vc units. The ensuing adaptation or desensitization in Vc firing may again be mediated via peripheral and/or central effects. Peripherally, adaptation or desensitization of nociceptor firing might be due to desensitization of nAChRs in the nociceptor endings, or to a secondary process triggered by influx of Ca2+ through the nAChR- or voltage-gated ion channels (Holladay et al. 1997) to reduce cellular excitability (see Introduction). Repeated application of nicotine elicited inward currents in trigeminal ganglion cells that decreased in amplitude across trials at a 3-min ISI (Liu and Simon 1996a), consistent with a peripheral site of adaptation/desensitization. Alternatively, reduced Vc firing in the presence of nicotine might be mediated by a central process such as reduced transmitter release from primary afferents, although this seems less likely. In any event,
adaptation/desensitization appears to overcome the excitatory
effect of nicotine, in contrast to capsaicin for which the reverse
holds.

The present results are consistent with previous psychophys-
ical studies showing a progressive reduction in nicotine-evoked
irritation across trials (Dessirier et al. 1997, 1999a). The initial
nicotine stimulus excited Vc units within seconds (Figs. 10 and
11) (Carstens et al. 1998), consistent with the onset of irritant
sensation. Interestingly, Vc firing increased further in the sec-
ond and third minute of stimulation before declining (Figs. 10B
and 11B), whereas human ratings already began to decline with
This difference might be attributed to the higher concentration
and/or volume of nicotine used presently (10% in 0.1 ml)
compared with the smaller dose (0.12% in 35 μl) given by
filter paper in the psychophysical studies (Dessirier et al. 1997,
1999a). Thus in the present study the more concentrated nicoci-
ne may have diffused further to recruit additional nocicep-
tors during the initial 3 min of application before adaptation/
desensitization set in. In the human studies, the less-concentrated
nicotine, applied over a restricted area, did not provide
as much spatial summation and thus allowed adapt-
ation/desensitization to manifest more quickly.

In conclusion, the present study has revealed contrasting
patterns of Vc unit firing that are consistent with, and might
underlie, the respective sensitization and desensitization in the
irritant sensations elicited by repeated application of capsaicin
compared with nicotine.

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