

# Time Course of Self-Desensitization of Oral Irritation by Nicotine and Capsaicin

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## Abstract

Nicotine contacting mucous membranes elicits irritation that decreases with repeated exposures (self-desensitization). We investigated the time course of nicotine self-desensitization and compared it with that of capsaicin. Nicotine (300 mM, 10  $\mu$ l) was applied to one-half of the dorsal tongue and vehicle to the other. Following a rest period ranging from 0.5 to 48 h, nicotine (5  $\mu$ l) was reapplied to each side of the tongue and subjects indicated on which side they experienced stronger irritation and separately rated the intensity of the sensation on each side. After intervals of 0.5, 1, and 24 h, a significant majority of subjects chose the vehicle-treated side as having stronger irritation and assigned significantly higher intensity ratings to that side, indicating self-desensitization. The effect was not present after 48 h. By comparison, 10 parts per million (ppm) (33  $\mu$ M) capsaicin induced significant self-desensitization at 1 but not 24 h, whereas a higher concentration of capsaicin (100 ppm, 330  $\mu$ M) induced significant self-desensitization at intervals of 1, 24, and 48 h. These results indicate that initial exposure to nicotine or capsaicin can markedly attenuate irritant sensations elicited by subsequent exposure to these irritants hours to days later.

**Key words:** 2-alternative forced choice, capsaicin, nicotine, oral irritation, psychophysics, self-desensitization

## Introduction

Nicotine, a bioactive constituent of tobacco, elicits a burning irritation upon contact with oral or ocular mucosa (Keele and Armstrong 1964). In human psychophysical studies, the magnitude of lingual nicotine-evoked oral irritation decreases across trials of repeated application at 1-min intervals (Dessirier et al. 1997, 1999), a phenomenon commonly referred to as desensitization. Lingual nicotine excites nociceptive neurons in trigeminal subnucleus caudalis (Vc) in a temporally similar desensitizing pattern (Dessirier, Simons, et al. 2000). After a rest period, reapplication of nicotine elicits significantly less irritant sensation (Dessirier et al. 1997) and Vc neuronal firing (Dessirier, Simons, et al. 2000; Sudo et al. 2002), often referred to as self-desensitization. With low millimolar nicotine concentrations, recovery of nicotine irritation occurred within 5–10 min (Dessirier et al. 1997); at higher (300–600 mM) concentrations, recovery of nicotine-evoked Vc neuronal responses was not observed after 1 h (Sudo et al. 2002). In prior (Dessirier, Chang, et al. 2000) and ongoing studies, it was advantageous to deliver nicotine in fairly high concentration and low volume to elicit a moderate level of oral irritation without central effects. We presently investigated the time course of self-

desensitization of oral irritation elicited by this level of nicotine. We were particularly interested in comparing nicotine with capsaicin, which is well known to induce self-desensitization (Szolcsanyi 1977; Green 1989; Karrer and Bartoshuk 1991; Dessirier et al. 1997). We therefore also investigated the time course of self-desensitization by capsaicin at 2 concentrations (10 and 100 ppm; 33 or 330  $\mu$ M) used commonly in human psychophysical studies of oral irritation.

## Methods

All experiments were approved by the UC Davis human subjects committee. A total of 279 subjects (182 females, 97 males; 18–55 years of age) consisting of staff, faculty, and students at UC Davis participated in one or more of the experiments. Subjects were recruited through the Experimentrix online scheduling Web site (<http://www.experimentrix.com/ucdavis>). All subjects were self-reported nonsmokers and were instructed to refrain from eating spicy food 48 h prior to the experiment and to refrain from using mentholated products 1 h prior to the experiment.

The half-tongue, 2-alternative forced-choice (2-AFC) method was similar to that used in previous experiments (Simons et al. 2002; Simons et al. 2003b). Subjects first rinsed the mouth with distilled water. A filter paper (1.5 cm diameter, 176.7 mm<sup>2</sup>; Whatman, Maidstone, UK) containing a 10 µl volume of nicotine (free base; 300 mM [5%] in distilled water; Sigma Chemical Co., St Louis, MO) was then applied with forceps onto one side of the anterior dorsal surface of the tongue, and another filter paper with 10 µl distilled water was applied at the mirror image location on the other side. After 15 s, the filter papers were removed and subjects asked to rate the intensity of irritation on each side of the tongue using a 0–10 visual analog scale with 0 = no irritation and 10 = most intense irritation imaginable. The high concentration and low volume was chosen because it elicits moderate irritation while limiting the total nicotine dose to 0.5 mg. The side receiving nicotine was randomized across subjects. After a waiting period of 0.5, 1, 24, or 48 h, the subject returned and smaller filter papers (1 cm diameter, 78.5 mm<sup>2</sup>; Whatman), each with 5 µl of nicotine, were placed with forceps simultaneously onto each side of the tongue within the region that had earlier received the larger filter paper stimulus. Generally, separate groups of subjects were tested at each interstimulus interval with the following exceptions: 1) one group of 32 subjects was tested with nicotine at both 0.5 and 1 h interstimulus intervals in separate sessions at least a week apart, and 2) a separate group of 30 subjects was tested with 10 ppm capsaicin at both 0.5 and 1 h interstimulus intervals in separate sessions at least a week apart. In a 2-AFC paradigm, subjects were then asked to state on which side of the tongue they experienced a stronger irritant sensation. After the 2-AFC, subjects were asked to separately rate the intensity of irritation on each side of the tongue using the 0–10 rating scale.

The same procedure was followed with capsaicin at 10 ppm (33 µM) or 100 ppm (330 µM). These concentrations were chosen because they elicit low to moderate levels of irritation, respectively, and are commonly used in psychophysical studies of oral irritation. Also, the 100 ppm capsaicin concentration elicited a level of irritation that was comparable to that evoked by nicotine. Larger filter papers (1.5 cm diameter) were wetted with 40 µl of the 10 or 100 ppm capsaicin (diluted with distilled water from a stock solution of 0.1% capsaicin [98–100% pure; Sigma Chemical Co.] in 50% ethanol and water) and air dried to allow evaporation of ethanol in the solvent. Just prior to application to the tongue, the filter paper was wetted with 40 µl of distilled water and placed onto one side of the dorsal anterior tongue using forceps for 15 s and then removed. A second filter paper wetted with 40 µl of capsaicin vehicle was applied simultaneously to the opposite side in the same manner. After unilateral application of 10 ppm capsaicin, subjects returned 0.5, 1, or 24 h later to receive bilateral application of 10 ppm capsaicin. Smaller (1 cm diameter) filter papers were prepared as above by soaking them with 20 µl of 10 ppm capsaicin, air drying,

and rewetting with water just prior to bilateral application to the same areas of the tongue that had previously received the larger filter papers. After 15 s, both filter papers were removed, followed by the 2-AFC and bilateral intensity ratings as above. Following unilateral application of 100 ppm capsaicin, subjects returned 1, 24, or 48 h later for bilateral application of 100 ppm capsaicin.

For 2-AFC data, the binomial test was used to determine if a significant proportion of subjects chose the vehicle-pretreated side of the tongue as having stronger irritation. We tested approximately 30 subjects under each condition because the binomial distribution approaches a normal distribution with this number of observations. Intensity ratings for the nicotine pretreated (or capsaicin pretreated) and vehicle-treated sides of the tongue were compared using the paired *t*-test. For both tests,  $P < 0.05$  (2-tailed) was considered significant.

## Results

### Nicotine

Upon initial application, ratings of irritation were significantly higher ( $P < 0.001$ ) for the nicotine-treated side compared with vehicle-treated sides (Table 1). Initial ratings were similar for the 0.5 and 1 h interstimulus interval conditions in which the same subjects were tested and were somewhat higher for the 24 and 48 h intervals that used separate groups of subjects.

When nicotine was applied bilaterally 30 min after unilateral lingual application of nicotine, a significant majority of subjects chose the vehicle-pretreated side (i.e., not pretreated with nicotine) as having stronger irritation in the 2-AFC (Figure 1A) and assigned significantly higher intensity ratings to that side (Figure 1B). Similar results obtained for 1 and 24 h interstimulus intervals, albeit with a progressive decrease in the percentage of subjects choosing the vehicle-treated side in the 2-AFC (Figure 1A). The self-desensitization dissipated after a 48-h interstimulus interval as evidenced by a lack of significance in the 2-AFC and intensity ratings difference (Figure 1A,B).

### Capsaicin

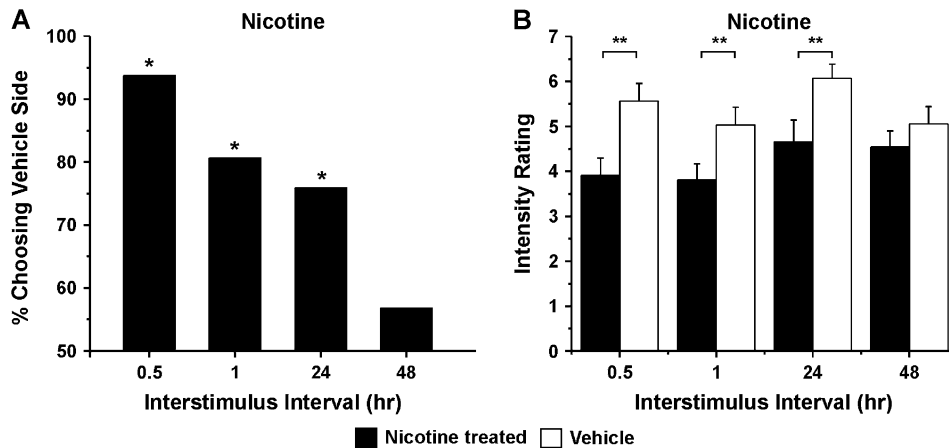
Upon initial application, irritant ratings were significantly ( $P < 0.001$ ) higher on the side treated with either 10 or 100 ppm capsaicin compared with the respective vehicle-treated sides (Table 1). Ratings for 100 ppm capsaicin were generally comparable to those for nicotine although there was some between-group variability.

When 10 ppm (33 µM) capsaicin was applied bilaterally either 0.5 or 1 h after its unilateral application, a significant majority of subjects chose the vehicle-pretreated side as having stronger irritation (Figure 2A) and gave significantly higher intensity ratings for that side (Figure 2B). The effect was gone after 24 h (Figure 2A,B). When 100 ppm (330 µM)

**Table 1** Initial ratings of irritation under each experimental condition

Interstimulus interval (h)	Nicotine (300 mM)		Capsaicin 10 ppm		Capsaicin 100 ppm	
	T	Veh	T	Veh	T	Veh
0.5	5.18 (0.31)	1.59 (0.29)	3.87 (0.37)	0.4 (0.11)	—	—
1	5.26 (0.33)	0.84 (0.21)	3.85 (0.41)	0.57 (0.19)	—	—
24	6.89 (0.32)	1.03 (0.22)	4.27 (0.39)	0.6 (0.19)	5.87 (0.33)	1.61 (0.26)
48	6.05 (0.39)	0.73 (0.2)	—	—	5.06 (0.35)	1.03 (0.24)

T: nicotine- or capsaicin-treated side; Veh: vehicle-treated side. Numbers in parentheses: standard error of mean. —: not tested.



**Figure 1** Nicotine self-desensitization. **(A)** 2-AFC results. Nicotine was applied to one side of the tongue, followed by a waiting period of 0.5, 1, 24, or 48 h. After the waiting period, nicotine was applied to both sides of the tongue and subjects stated on which side the irritant sensation was greater. \*: Significant majority chose the side not previously treated with nicotine ( $P < 0.05$ , binomial test;  $n = 30$  or more for each interval). **(B)** Bilateral intensity ratings. After performing the 2-AFC, subjects independently rated the intensity of irritation on each side of the tongue using a 0–10 scale with 0 = no irritation and 10 = strongest irritation imaginable. Error bars in this and subsequent figures: SEM. \*\*: Significant difference between ratings on previously nicotine-treated versus untreated side,  $P < 0.01$ , paired  $t$ -test.

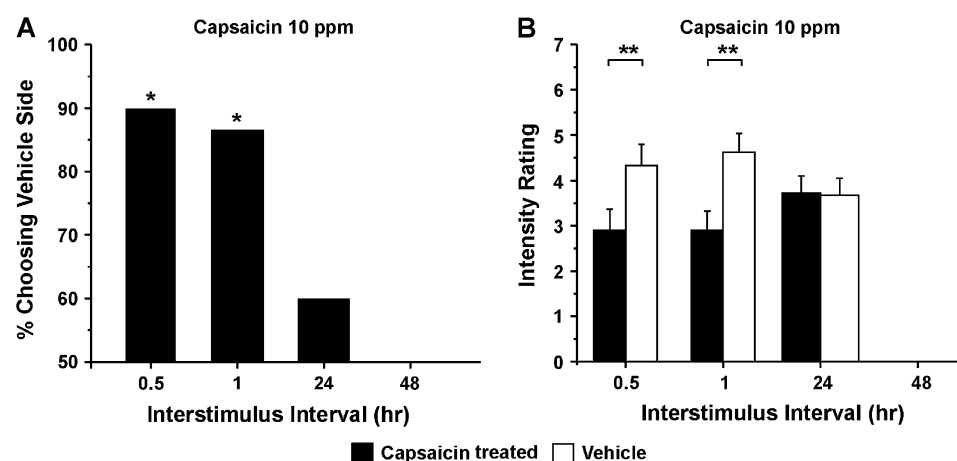
was similarly applied, a significant majority of subjects chose the vehicle-treated side as having stronger irritation (Figure 3A) and assigned significantly higher intensity ratings to that side (Figure 3B) at all interstimulus intervals out to 48 h.

## Discussion

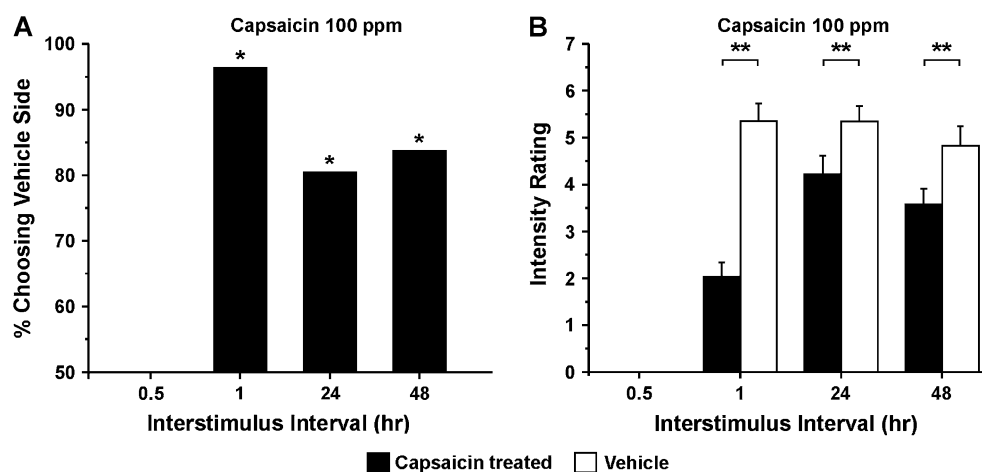
Self-desensitization of the oral irritant sensation of nicotine persisted for 24 h and was no longer significant after 48 h, indicating that if desensitization were still present the effect was relatively small. From an experimental standpoint, these results indicate that a 2-day interval between testing sessions is sufficient to avoid a significant carryover effect of nicotine, although additional studies would be needed to determine if the effect is completely absent. From a practical viewpoint, the oral ingestion of products that contain enough nicotine to induce irritation, such as tobacco smoke, chewing tobacco, or nicotine gum, is likely to induce prolonged self-desensitization. A reduction in irritation lasting longer than

24 h might lead in turn to heavier daily consumption of the nicotine-containing product because the warning signal provided by the irritant sensation is diminished. In this setting, it is not known to what extent resumed consumption of nicotine overcomes desensitization to result in stimulus-induced recovery (SIR) of irritancy that has been observed with capsaicin (Green and Rentmeister-Bryant 1998). If nicotine exhibits SIR, then its self-desensitizing effect would serve to delay future nicotine-evoked irritation rather than causing a prolonged loss of sensitivity.

The mechanism of nicotine self-desensitization is not clear but might involve a local anesthetic effect on nociceptive trigeminal afferents in the oral cavity via nonspecific inhibition of tetrodotoxin-resistant voltage-gated sodium channels (Liu et al. 2004). Alternatively, it may involve desensitization of nociceptive nerve endings in the oral mucosa via a neuronal nicotinic acetylcholine receptor (nAChR)-mediated mechanism. Nicotine-evoked irritation (Jarvik and Assil 1988; Dessirier et al. 1998) and excitation of Vc neurons (Carstens et al. 1998, 2000) are significantly attenuated by



**Figure 2** Self-desensitization following 10 ppm (33  $\mu$ M) capsaicin (format as in Figure 1).



**Figure 3** Self-desensitization following 100 ppm (330  $\mu$ M) capsaicin (format as in Figure 1).

the nAChR antagonist mecamylamine. Nicotine at high concentrations cross-desensitized capsaicin-evoked irritation (Dessirier, Chang et al. 2000) and Vc neuronal responses to pentanoic acid, the latter effect being prevented by mecamylamine (Simons et al. 2003b). We speculate that nicotine self-desensitization may involve a similar nAChR-mediated mechanism at mucosal nerve endings of nociceptors. However, we cannot rule out the possibility that the initial nicotine stimulus activates central mechanisms to reduce transmission in pain pathways that are activated by subsequent nicotine.

Repeated lingual application of capsaicin at 1-min interstimulus intervals elicited a progressive rise in intensity ratings of irritation that is referred to as sensitization (Green 1989; Dessirier et al. 1997). Imposition of a rest period of 2.5–5 min resulted in self-desensitization (Green 1989). In a subsequent study, Karrer and Bartoshuk (1991) reported that self-desensitization of lingual irritation elicited by 10

or 100 ppm capsaicin recovered within 1–2 and 2–4 days, respectively. When a concentration series of capsaicin (up to 1000 ppm) was applied, self-desensitization did not recover after 6 days (Karrer and Bartoshuk 1991). Our present data are in good agreement. Capsaicin (10 ppm [33  $\mu$ M]) produced significant self-desensitization after 1 but not 24 h, whereas 100 ppm (330  $\mu$ M) resulted in prolonged self-desensitization that had not recovered by 48 h. The somewhat shorter duration of self-desensitization to 10 ppm capsaicin observed presently might be attributable to methodological differences; we applied a lower volume of capsaicin by filter paper compared with the Karrer and Bartoshuk (1991) study in which capsaicin was applied by swab to the whole anterior tongue. The longer time courses of self-desensitization observed with 100 ppm capsaicin and 300 mM nicotine suggests that irritant potency plays a role in the duration of the effect. Presently, we did not systematically match the relative irritant intensities, although 100 ppm capsaicin and 300 mM nicotine were

reasonably well matched for irritant intensity (Table 1). Ratings for capsaicin tended to be lower (Table 1) and the duration of self-desensitization was longer compared with nicotine (Figures 1–3), suggesting that the duration of self-desensitization may depend on factors in addition to relative irritant intensity. Overall, the data show that the duration of capsaicin self-desensitization is dose dependent, a finding of relevance to consumption of spicy foods, beverages, and oral hygiene products containing irritant chemicals. For example, frequent consumers of red chili have been reported to exhibit somewhat higher detection thresholds and lower intensity ratings for capsaicin compared with infrequent consumers (e.g., Rozin et al. 1981; Lawless et al. 1985; Stevenson and Prescott 1994), possibly reflecting chronic desensitization due to regular ingestion of sizable amounts of capsaicin.

The mechanism underlying capsaicin self-desensitization likely involves a reduction in excitability of peripheral nociceptor nerve endings via activation of transient receptor potential V1 channels (Cholewinski et al. 1993) to initiate intracellular calcium-dependent (Cholewinski et al. 1993; Liu and Simon 1996a, 1996b) and calcineurin-dependent (Docherty et al. 1996) events leading to a reduction in membrane excitability. Another potential mechanism involves capsaicin inhibition of voltage-gated sodium channels to suppress the generation of action potentials in nociceptive nerve endings (Liu et al. 2001).

The current findings are relevant to industrial settings where foods and consumer products containing irritant chemicals are routinely evaluated. Many sensory protocols ask panelists to evaluate multiple items and require them to take a break between samples when the products contain oral irritants. The present results suggest that desensitization may still be present even after a 24- to 48-h hiatus. New sensory paradigms will need to be developed that attend to the temporal aspect of desensitization and the extent to which it can be overcome by SIR.

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