Spatial and Temporal Effects of Capsaicin and Menthol on Intraoral Somatosensory Sensitivity

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Aims: To assess the spatial and temporal sensory effects of the topical application of capsaicin and menthol on the gingiva of healthy volunteers. **Methods:** Capsaicin, menthol, and saline (control) were applied topically on the gingiva in the maxillary premolar area of healthy volunteers for 15 minutes. Pain intensity was rated on a 0 to 10 visual analog scale (VAS). Before, immediately after, and 30 minutes after application, three mechanical stimuli were applied at 15 gingival sites: fixed-intensity stimuli were applied by 32 mN and 512 mN von Frey filaments, and stimuli of increasing intensity were applied by an electronic von Frey (EVF, 10 g/s). The EVF was used to test the pinprick pain threshold (PiPT). The perceived pain from filament stimulation was rated on a 0-50-100 numeric rating scale (NRS). Analysis of variance for repeated measures was used to analyze the NRS scores, PiPT values, the number of hypersensitive or hyposensitive test sites, and the coordinates of the center of gravity (COG) of somatosensory sensitivity. **Results:** The mean ± SEM VAS score of pain intensity produced by the application of capsaicin (4.6 ± 0.5) was significantly higher than that produced by menthol (0.3 ± 0.2) and saline (0.1 ± 0.1) ($P < .001$). Capsaicin induced local desensitization to all stimuli ($P < .047$), and at the application site, capsaicin induced significant desensitization to 512 mN stimuli ($P = .003$). Menthol did not induce significant somatosensory changes ($P > .147$), and saline induced slight desensitization in two sites surrounding the application site ($P < .023$). The COG coordinates did not shift significantly over time during any condition ($P > .125$). **Conclusion:** Capsaicin but not menthol induced mechanical desensitization in the application area but not in the surrounding areas. *J Oral Facial Pain Headache 2015;29:257–264. doi: 10.11607/ofph.1106*

Keywords: *capsaicin, intraoral somatosensory testing, menthol, psychophysics, spatial, temporal*

Experimental pain models can provide insights into changes in somatosensory sensitivity and may be important for the study of mechanisms of neuropathic pain.[1–3] Capsaicin and menthol are two algesic chemicals commonly used in surrogate models of heat and cold hyperalgesia.[4–8]

Previous cutaneous studies have demonstrated that the sensory alteration induced by capsaicin or menthol application occurs not only in the zone of application (primary zone) but also in the area surrounding the primary zone (secondary zone).[7,9] Based on these studies, it has been hypothesized that experimentally induced hyperalgesia in the primary zone is a result of primary afferent nociceptor activation and sensitization, while the sensitization of neurons in the central nervous system results in mechanical hyperalgesia in the secondary zone.[10] Furthermore, these studies have demonstrated two types of secondary hyperalgesia: one is enhanced pain to pinprick stimuli (punctate hyperalgesia), and the other is pain to light touch (allodynia).[11,12] The secondary punctate hyperalgesia represents facilitation of one nociceptive input (capsaicin-insensitive high-threshold A-fiber mechanoreceptors)

by another nociceptive input (eg, capsaicin-sensitive C-fiber nociceptors), while secondary allodynia represents a crosstalk of A-fiber low-threshold mechanoreceptive input into nociceptive pathways.[9,12,13]

Intraoral studies have shown that the sensitivity of the gingiva is different from that of the skin to the application of capsaicin and menthol. For instance, studies performed on the skin showed that capsaicin increases the sensitivity to mechanical stimulations of both the primary and secondary zones.[4,7,9] On the contrary, the application of capsaicin to the gingiva resulted in a mechanical desensitization in the application area (primary zone)[5,14]; the mechanisms behind such a sensory alteration are still unclear. Moreover, the topical application of menthol induced both primary and secondary mechanical pinprick hyperalgesia on hairy skin[7] but not on the gingiva.[14]

The temporal and spatial aspects of the sensory changes after capsaicin application to the attached gingiva have not been studied systematically, mainly due to the difficulties in precise and reproducible location of the test sites intraorally. It is only recently that an intraoral template has been developed that allows investigation of the spatial variation in intraoral somatosensory sensitivity.[15] Thus, the present study used this template to assess the spatial and temporal sensory effects of the topical application of capsaicin and menthol to the gingiva of healthy volunteers. The hypothesis of the study was that capsaicin and/or menthol alters the somatosensory sensitivity to mechanical stimuli in the primary and secondary zones in comparison with a saline control.

Materials and Methods

Subjects

A total of 15 healthy volunteers (6 male and 9 female) with a mean ± standard error of the mean (SEM) age of 26.1 ± 1.4 years were recruited by posting an advertisement on www.forsoegsperson.dk and at Aarhus University. All volunteers reported to be in good general and oral health. The exclusion criteria were a history of orofacial pain and painful temporomandibular disorders (TMD) according to the Research Diagnostic Criteria for TMD (RDC/TMD),[16] the presence of oral mucosal lesions, pregnancy, mental disorders, hypochondria, dental treatment scheduled for the time of study, intake of medication within 48 hours of the investigation (analgesics, antidepressants, or hypnotics), and allergy to capsaicin or menthol. The study protocol was approved by the Local Ethics Committee (Central Denmark Region, Denmark), and written informed consent was obtained from all participants. The study was conducted in accordance with the Declaration of Helsinki.

Study Design

The study was performed in a randomized, double-blind, placebo-controlled crossover manner. The full procedure included two sessions separated by 3 to 7 days to avoid possible carryover effects. Capsaicin and isotonic saline were applied in one session; menthol and saline were applied in the other session. The sequence of the two sessions and the order of applications of the two substances, as well as the side of application (right or left side), were randomly chosen.

The examiner applied 30 µL of 5% capsaicin, 40% menthol, or saline (control) on a 3 × 3-mm paper disk under a carefully applied and fitted oral bandage (Urihesive, ConvaTec) on the buccal aspect of the attached gingiva cranially to the maxillary first premolar for 15 minutes.[5] In this way it was possible to prevent the capsaicin, menthol, or saline from spreading to nearby regions or into the oral cavity.[5] The concentration and the application period were chosen based on earlier studies.[5,7,14] Subjects were asked to score their real-time perceived pain intensity on a 0 to 10 electronic visual analog scale (VAS) by sliding a knob between the two VAS extremes "no pain" and "worst pain imaginable."[17] The electronic VAS signal was sampled in 1-second intervals during the entire 15-minute period by the computer program. Quantitative sensory testing (QST) was performed before, immediately after, and 30 minutes after removal of the oral bandage in the same sequence. Thereafter, the same procedure was repeated on the opposite side. All subjects and the examiner were blind to the substances, which were prepared by a research assistant. All participants were tested in a quiet room at normal room temperature by the same female examiner.

Somatosensory Tests

Left and right intraoral templates made of impression material (President, Coltene) were constructed for each subject.[15] The same templates were used in both sessions. The templates that were used only for the QST covered approximately 20 × 10 mm of the buccal aspect of the mucogingival tissue. The center of each template (capsaicin/menthol/saline application site, ie, primary zone) corresponded to the buccal gingiva above the first maxillary premolar, extending from the attached gingiva to the mucobuccal fold[15] (Fig 1a).

Each template had 15 equidistant holes (diameter approximately 2 mm) in a 3 × 5 matrix (distance between two adjacent holes of 5 mm). The 14 holes surrounding the central hole were considered representative of the secondary zone.

Quantitative Sensory Testing

Two different techniques were used to assess the somatosensory sensitivity. First, a response-dependent

(magnitude estimation) psychophysical technique was used for assessment of tactile and pinprick sensitivity.[18] The tactile sensitivity was assessed by applying a 32-mN force and the pinprick sensitivity by applying a 512-mN force by means of von Frey optic glass fiber filaments (OptiHair, Marstock Nervtest). In the standardized QST protocol proposed by the German Research Network for Neuropathic Pain, mechanical pain sensitivity is assessed using a group of custom-made weighted pinprick stimulators,[19] while in the present study, a 512-mN von Frey filament with a round tip was used as the pinprick stimulus, for the following three reasons: *(1)* intraoral mucosal tissues differ from cutaneous tissues, ie, the loosely bound oral mucosa is more fragile than skin, and a pilot study showed that after repetitive use of pinprick stimulators, some mucosal sites (especially those closer to the mucogingival fold) were irritated and a few even bled; *(2)* the pinprick stimulators require a vertical load to all test sites, which was not possible at all sites due to the limited intraoral space; and *(3)* based on a previous study, the intraoral mean mechanical detection and pain thresholds are about 36.2 mN and 242.8 mN, respectively,[14] thus 32 mN and 512 mN filaments were applied in order to assess innocuous (tactile) and nociceptive functions. The filament was inserted in each template hole in random order[15] (Fig 1). Each test site was stimulated for about 1 to 2 seconds,[19,20] and the stimulation was repeated three times consecutively.

The subjects scored the intensity of the sensation produced by the mechanical stimuli on a 0-50-100 numeric rating scale (NRS) with 0 denoting "no sensation at all," 50 as "just barely painful," and 100 as "the most imaginable pain."[21] This NRS was chosen to encompass both nonpainful and painful ratings and has been used extensively.[21–23] The subjects were asked to give an average NRS score for the three stimuli, and this average was used for further analysis. This approach was chosen for practical reasons and to minimize movement of the lips.

The second psychophysical technique used to assess the pinprick pain threshold (PiPT in grams) used a classical threshold detection protocol.[15] The threshold was determined on both sides and at each test site by means of an electronic von Frey stimulator (EVF, SENSEbox, Somedic).[15] The EVF pressure intensity was increased at a constant rate of 10 g/s. The EVF, which had a rounded tip with the diameter of 0.2 mm, was applied in random order between the 15 sites. The subject pushed a button as soon as he or she felt the slightest painful sensation. One threshold per test site was assessed.

The entire procedure lasted approximately 15 minutes. The three stimuli were always applied in the same order: 32 mN, 512 mN, and EVF.

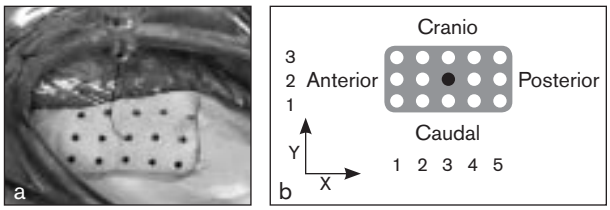

Fig 1 **(a)** Template in situ. **(b)** Coordinates (X = anteroposterior direction; Y = craniocaudal direction). The black circle (X = 3, Y= 2) corresponds to the area where capsaicin, menthol, and saline were applied.

Statistical Analyses

The NRS scores and PiPT values were analyzed in three steps. First, the NRS scores and PiPT values recorded at the 15 sites at baseline (over four sessions) were averaged to obtain an overall assessment of the somatosensory sensitivity. Second, the 95% confidence interval (CI) was determined. The sites at which the NRS score was above the 95% CI were defined as hypersensitive sites, and those below 95% CI were defined as hyposensitive sites. The numbers of hypersensitive and hyposensitive sites were counted for each substance. Finally, the center of gravity (COG) coordinates of somatosensory sensitivity were calculated.[24] COG was defined as ΣXi^* grid value / Σ grid value. The NRS scores and PiPT values were used as the grid value.[15,24,25] The site coordinates (X = anteroposterior direction, Y = craniocaudal direction) (Fig 1b) were used as the "i" when calculating the COG. Hence the COG represents the position of the center of the weighted average of the NRS or PiPT values.

Data are presented as mean values ± SEM. Since comparisons of each baseline pair of NRS scores and PiPT for all three stimulus modalities from contralateral sites with the same anteroposterior and craniocaudal location showed no significant differences, three-way repeated-measures analyses of variance (ANOVA) were used to test differences in NRS scores and PiPT values with the following factors: application (3 levels—capsaicin, menthol, control), time (3 levels—baseline, immediately after, 30 minutes after), and test site (15 levels). Direct post-hoc tests (Tukey Honestly Significantly Different [HSD] test) were used to compare differences in NRS and PiPT values between each timepoint at each site. Differences in the number of hypersensitive or hyposensitive test sites and COG coordinates were tested by two-way repeated-measures ANOVA with application (three levels) and time (three levels) as factors. The spatial variation in intraoral somatosensory sensitivity before application has being published previously.[15] For all tests, a $P < .05$ was considered to be statistically significant.

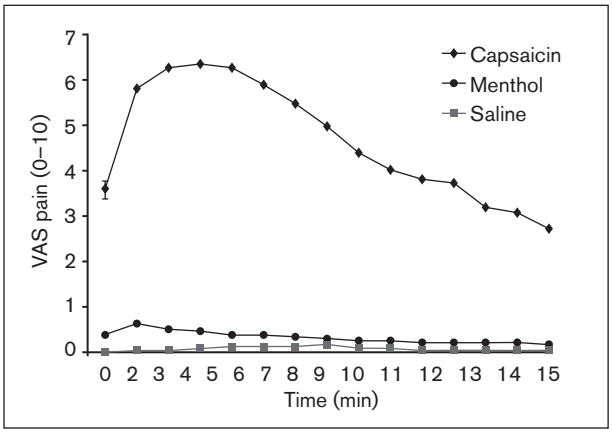

Fig 2 Variations over time of the mean VAS pain scores of 15 participants during each minute for capsaicin, menthol, or saline application (*P < .050; **P < .001).

Table 1 Overall Mean Values (±SEM) of 15 Test Sites of Three Different Stimulus Modalities and *P* Values for Time and Application Effects (three-way ANOVA)

	32 mN	512 mN	EVF
Capsaicin			
Baseline	12.5 (1.8)	34.3 (2.6)	31.9 (2.8)
Immediately after	8.3 (1.6)	27.5 (2.4)	35.3 (3.1)
30 min after	10.7 (1.5)	28.9 (2.4)	32.0 (2.4)
Menthol			
Baseline	10.5 (1.5)	31.6 (2.6)	30.4(2.3)
Immediately after	9.1 (1.3)	30.9 (2.7)	30.2 (2.4)
30 min after	9.6 (1.3)	29.2 (2.6)	27.9 (2.4)
Saline			
Baseline	11.7 (1.5)	36.4 (2.5)	33.7 (3.0)
Immediately after	10.5 (1.7)	33.1 (3.2)	33.0 (2.9)
30 min after	9.8 (1.5)	30.4 (2.8)	31.4 (2.8)
***P* value**			
Application	.914	.811	.627
Time	**.002**	**.000**	**.097**
Site	**.000**	**.000**	**.000**

EVF = electronic von Frey.

RESULTS

Perceived Pain Intensity

All participants scored the application of capsaicin as moderately painful. The mean peak pain intensity produced by capsaicin (7.3 ± 0.6) was significantly higher than that caused by menthol (1.2 ± 0.3) and saline (0.3 ± 0.2) (P < .001).

The overall mean pain evoked by capsaicin (4.6 ± 0.5) was also significantly higher than that caused by menthol (0.3 ± 0.2) and saline (0.1 ± 0.1) applications (P < .001).

The variations of pain scores (mean value ± SEM) of the 15 participants at each minute over a 15-minute period for capsaicin, menthol, or saline are shown in Fig 2.

Quantitative Sensory Testing

Overall, the NRS scores for the 32-mN and 512-mN stimuli did not vary significantly among the different substances that were applied to the gingiva (P > .811) but did vary significantly between timepoints and sites (P < .002). The post-hoc tests showed that the NRS scores after application were significantly lower than before application for the 32-mN stimulus (P < .002). For the 512-mN stimulus, this was the case both immediately and 30 minutes after application (P = .000). The PiPT values did not vary significantly among the different substances and timepoints (P > .097) but did vary significantly between sites (P = .000) (Table 1).

Application of Capsaicin

The NRS scores and PiPT values varied significantly between timepoints and sites both for the 32-mN and 512-mN stimuli (P < .019 and P < .009, respectively) and PiPT (P < .046).

The post-hoc tests showed that the NRS scores obtained immediately after capsaicin application were significantly lower than at baseline for both the 32-mN and 512-mN stimuli (P < .015), while at 30 minutes post-application this was the case only for the 512-mN stimulus (P < .047).

The post-hoc tests showed that the NRS scores recorded with the 32 mN stimulus and PiPT values did not differ significantly over time at any individual test site (P > .295; P > .121, respectively), although at site (X = 3, Y = 2) (primary zone), a statistically significant decrease in sensitivity to stimulation with 512 mN was detected immediately after application compared with before application (P = .003) (Fig 3).

Application of Menthol

The NRS scores obtained with the 32-mN and 512-mN stimuli and the PiPT values varied significantly between sites (P < .001) but not over time (P > .147) (Fig 3).

Application of Isotonic Saline

The NRS scores obtained with the 32-mN stimulus and the PiPT values varied significantly between sites (P < .001) but not over time (P > .252). The NRS scores registered with the 512-mN stimulus varied significantly between timepoints and sites in the saline session (P < .001).

The NRS scores obtained immediately and 30 minutes after saline application were significantly lower than at baseline (P < .004). The post-hoc tests showed significantly decreased NRS scores immediately after application at two sites (X = 2, Y = 2; and X = 4, Y = 2) (secondary zone) (P < .023) and 30 minutes after application at another site (X = 1, Y = 2) (P = .019) (secondary zone) compared with before application (Fig 3) (Table 2).

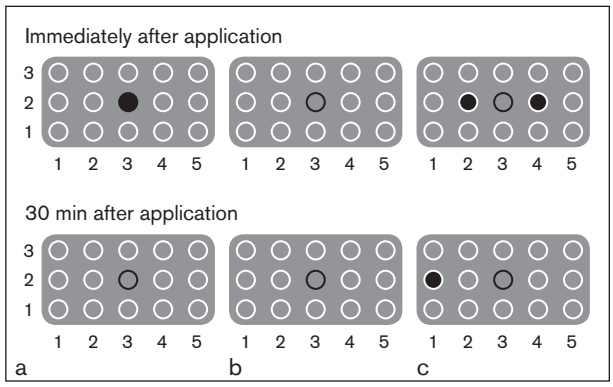

Fig 3 Comparison of the spatial effects caused by the three substances immediately after application and 30 minutes after application. **(a)** At 30 minutes after capsaicin application, the numeric rating scale (NRS) scores decreased (black circle) at site (3, 2) for the 512-mN stimulus (P = .003). **(b)** No NRS scores or pinprick threshold (PiPT) values varied significantly over time after menthol application. **(c)** Immediately after saline application, the NRS scores decreased (black circles) at site (2, 2) and (4, 2) with the 512-mN stimulus (P < .023). At 30 minutes after saline application, the NRS scores decreased at site (1, 2) for 512-mN von Frey (P = .019).

Table 2 Mean NRS and PiPT Values of Three Different Stimulus Modalities at 15 Test Sites

	(1,3)	(1,2)	(1,1)	(2,3)	(2,2)	(2,1)	(3,3)	(3,2)	(3,1)	(4,3)	(4,2)	(4,1)	(5,3)	(5,2)	(5,1)
32 mN (NRS scores)															
Before capsaicin	23.6	15.1	4.9	27.0	13.4	6.9	20.5	11.6	7.0	16.8	8.8	6.3	13.9	7.7	4.1
After capsaicin	22.7	12.0	3.5	19.6	6.9	4.2	11.5	5.0	1.9	9.9	3.6	3.0	10.7	5.5	4.3
30 min after capsaicin	19.5	12.8	5.0	20.6	12.5	6.0	17.5	8.5	3.9	14.9	7.5	4.0	14.7	8.9	4.0
Before menthol	18.9	13.0	4.4	22.9	14.3	5.5	17.1	7.8	5.8	11.1	6.3	6.1	9.7	8.9	5.7
After menthol	13.7	16.0	4.0	17.8	8.0	4.1	16.5	7.6	5.7	13.3	5.9	4.1	9.3	6.8	3.5
30 min after menthol	16.3	12.0	4.1	18.9	14.1	6.5	17.5	6.9	4.9	12.7	8.0	4.3	7.6	8.0	2.8
Before saline	18.8	14.4	4.6	22.0	16.5	6.8	19.8	10.1	6.3	15.4	9.6	5.1	10.8	9.6	5.0
After saline	20.5	13.3	3.6	20.2	13.3	4.6	20.6	7.5	4.5	15.0	6.9	4.3	12.7	7.2	3.5
30 min after saline	20.9	13.4	3.7	16.8	14.7	5.7	16.2	7.5	3.8	13.3	6.3	4.2	11.8	6.0	3.2
512 mN (NRS scores)															
Before capsaicin	49.3	33.9	23.1	50.7	41.4	20.5	40.9	**38.8**	23.1	42.2	36.7	22.8	38.3	32.2	20.1
After capsaicin	46.7	30.1	16.1	39.7	29.9	20.0	35.3	**22.9**	18.9	34.5	24.3	17.6	34.0	27.7	15.0
30 min after capsaicin	47.0	34.2	16.5	37.9	36.0	18.7	35.6	30.7	17.1	34.9	29.1	18.9	32.3	28.5	15.5
Before menthol	45.9	37.9	17.3	45.5	36.5	24.6	41.2	31.5	15.9	36.3	32.7	19.9	36.7	32.7	18.8
After menthol	46.3	30.0	19.1	44.4	39.6	19.7	41.5	30.7	19.7	35.4	34.6	19.7	39.3	29.3	13.9
30 min after menthol	40.6	39.1	16.7	39.1	42.6	15.9	35.5	25.9	19.3	35.3	30.7	15.9	36.1	27.9	16.8
Before saline	48.9	**40.5**	25.8	50.4	**45.5**	28.4	45.9	38.9	27.1	40.0	**37.3**	24.8	40.9	34.5	17.0
After saline	47.6	51.1	20.7	48.6	**41.4**	21.2	44.9	34.1	21.9	38.9	**30.5**	17.2	38.3	29.0	11.3
30 min after saline	45.2	**33.3**	18.9	46.5	41.5	19.1	39.9	31.3	18.6	37.3	31.5	18.2	34.5	27.7	13.0
EVF (PiPT [g])															
Before capsaicin	22.8	26.0	43.2	19.1	23.4	44.4	22.9	19.6	38.7	33.4	21.3	46.0	33.1	31.5	52.5
After capsaicin	22.0	24.6	45.8	22.8	19.9	45.5	29.2	22.6	55.7	43.4	28.8	38.0	39.5	35.7	55.5
30 min after capsaicin	24.2	28.8	43.1	19.8	21.5	45.2	26.0	28.3	28.3	40.9	22.1	35.4	35.3	30.3	50.6
Before menthol	28.9	22.5	38.4	19.2	16.4	35.4	22.1	21.0	33.8	32.6	29.3	46.5	37.1	28.9	43.7
After menthol	29.7	23.6	39.6	19.5	16.8	39.1	23.3	19.0	41.5	31.4	21.0	43.2	36.6	30.1	39.0
30 min after menthol	25.5	22.5	35.3	18.7	17.1	32.0	24.2	20.4	33.3	34.5	10.3	43.7	32.7	29.5	39.5
Before saline	25.4	23.8	47.7	20.7	17.3	46.4	27.0	19.3	37.3	40.8	26.7	48.5	37.5	34.2	53.2
After saline	21.3	24.0	38.3	19.7	20.9	47.2	32.0	20.1	36.4	36.2	24.8	49.4	37.2	33.6	54.3
30 min after saline	20.2	23.5	37.4	19.2	19.9	43.3	25.2	19.6	37.0	35.1	23.0	46.0	36.7	31.3	54.3

NRS = numeric rating scale; PiPT = pinprick pain threshold; EVF = electronic von Frey.
Bold numbers: Significant within-session difference from before application.
Grey shading: Primary zone (application site, X = 3, Y = 2).

Number of Hyposensitive Test Sites

The number of hyposensitive sites recorded with the 32-mN and 512-mN stimuli did not vary significantly between the different substances (P > .145), but there was a significant change over time (P < .001) (Table 3). The post-hoc tests showed that the number of hyposensitive test sites significantly increased immediately after and 30 minutes after application both for 32-mN and 512-mN stimuli (P < .018). In a focused post-hoc comparison between substances at timepoints after application, the number of hyposensitive sites for 32-mN stimuli immediately after application (P = .040) and EVF at 30 minutes after application (P = .004) were also significantly different. The post-hoc tests showed that for 32-mN stimuli, capsaicin induced more hyposensitive test sites after its application than did saline (P = .039).

The number of hyposensitive sites revealed by PiPT values varied significantly between the different substances (P = .001) but not over time (P = .912)

Table 3 Mean number (± SEM) of Hypersensitive and Hyposensitive Test Sites of the Three Different Stimulus Modalities and *P* Values of Time and Application Effects (two-way ANOVA)

	32 mN		512 mN		EVF	
	Hyper	Hypo	Hyper	Hypo	Hyper	Hypo
Capsaicin						
Baseline	4.1 (0.2)	6.2 (0.8)	5.2 (0.4)	5.1 (0.3)	3.5 (0.3)	5.1 (0.3)
Immediately after	2.0 (0.5)	9.5 (0.8)	3.1 (0.6)	7.8 (1.0)	4.7 (0.6)	4.4 (0.6)
30 min after	3.5 (0.5)	7.3 (0.8)	3.7 (0.7)	6.8 (0.9)	3.7 (0.4)	3.9 (0.6)
Menthol						
Baseline	3.5 (0.3)	5.5 (0.7)	4.7 (0.4)	4.9 (0.4)	3.6 (0.4)	4.8 (0.3)
Immediately after	2.9 (0.5)	7.3 (0.9)	5.3 (0.8)	6.4 (1.0)	3.6 (0.4)	5.7 (0.4)
30 min after	3.5 (0.6)	7.3(1.0)	3.5 (0.9)	7.1 (1.0)	3.1 (0.4)	5.7 (0.4)
Saline						
Baseline	4.2 (0.4)	5.7 (0.4)	5.2 (0.2)	4.9 (0.3)	3.6 (0.2)	5.4 (0.2)
Immediately after	3.7 (0.5)	7.1 (0.5)	4.7 (0.6)	6.9 (0.6)	3.2 (0.2)	5.5 (0.4)
30 min after	3.5 (0.6)	7.1(0.6)	3.9 (0.5)	7.3 (0.6)	3.1 (0.3)	6.1 (0.4)
***P* value**						
Time	.056	**.001**	**.029**	**.000**	.210	.912
Application	.241	.145	.412	.777	.075	**.001**
Time × Application	.396	.484	.301	.817	.212	.083

Hyper = hypersensitive; hypo = hyposensitive; EVF = electronic von Frey.
Bold *P* values: statistically significant.

(Table 3). The post-hoc tests showed that the number of hyposensitive test sites after capsaicin application was significantly lower than after menthol or saline application ($P < .034$).

Number of Hypersensitive Test Sites

The number of hypersensitive sites registered with the 32-mN and 512-mN stimuli and the EVF did not vary significantly among the different applications ($P > .075$) but varied significantly over time for 512-mN von Frey ($P = .029$) (Table 3).

In a focused comparison between substances at timepoints after application, the number of hypersensitive test sites to EVF 30 minutes after application was significantly different ($P = .024$). The post-hoc test showed that capsaicin application ($P = .018$) but not menthol application ($P = .723$) induced more hypersensitive test sites than did saline application.

COG Coordinates

COG did not shift significantly between timepoints or substances (32-mN force: X = 2.7 ± 0.1, Y = 2.4 ± 0.1; 512-mN force: X = 2.9 ± 0.1, Y = 2.2 ± 0.0; and EVF: X = 3.1 ± 0.1, Y = 1.9 ± 0.0) ($P > .125$).

DISCUSSION

Perceived Pain

In the present study, topical application of capsaicin on the gingiva caused moderate levels of pain, whereas menthol application only caused mild levels of pain. The pain intensity was similar to that reported in previous studies in which 5% capsaicin or 40% menthol was applied to the same sites.[5,20] The duration of somatosensory changes induced by capsaicin and menthol were determined in the present experiment, and there was sufficient time in the present study to perform the three stimulus modalities of QST.

Quantitative Sensory Testing

Site-to-site differences in NRS scores and PiPT values at baseline have been reported elsewhere.[15] Generally, in the testing area (covered by the template), the region anterior and above (away from teeth) is more sensitive than the region posterior and below (close to teeth).[15] This is to be expected due to variations in thickness and degree of keratinization of the epithelium and the difference in hydration as well as nerve-fiber density between intraoral sites.

In the present study, three-way ANOVA for repeated measurements was used to compare changes in sensitivity between three timepoints but not to compare different sites within one timepoint. Therefore, the site-to-site differences within one timepoint did not influence the conclusions of this study.

Both static pinprick stimuli (512-mN von Frey filament) and dynamic pinprick stimuli (EVF) were used. Since the static pinprick stimuli were supposed to assess the response to supra-pain-threshold intensities, while dynamic pinprick stimuli were used for threshold determination, the two different stimulus protocols were employed to assess different aspects of pinprick sensitivity.[26] However, the 512-mN von Frey filament did not consistently evoke a painful sensation, which may be explained by the different physical properties of the stimulator tips. This can be considered a limitation of the present study. However, in addition, the

EVF was used as a complement to assess changes in the PiPT, which can be considered another aspect of mechanical pinprick sensitivity.[26]

In the present study, the subjects were asked to give an average score of the three NRS scores for von Frey filament stimuli instead of three single values for the following reasons: *(1)* it was difficult for the participants to speak with the template in place in the mouth; *(2)* to minimize the movement of the lips during testing. It would have been more ideal to obtain three individual values; therefore, this can be considered another limitation of the present study, although there is no evidence that the present methodology should be associated with less reliable or valid assessment of pinprick sensitivity.

Application of Capsaicin

A previous study has reported that high concentrations (> 1%) of capsaicin could lead to desensitization to mechanical stimuli.[27] In the present study, overall desensitization to mechanical stimuli, especially pinprick stimuli, was demonstrated after intraoral application of capsaicin. However, only the test site corresponding to the primary zone showed significant changes in pinprick (512-mN) sensitivity. The secondary zone did not show any significant change in the NRS scores or PiPT values in the post-hoc tests. The intraoral somatosensory changes in the primary zone were in accordance with those of previous studies[5,14] but contrary to those reported in studies of cutaneous sites.[9,11–12] Thus, capsaicin led to desensitization to pinprick stimuli only in the primary zone throughout the observation time. The finding that these changes were found only at the application site suggests that the attempt to avoid spread of the applied substances by the use of an oral bandage adhering to the gingiva was successful. However, no specific tests of the presence of substances outside the primary zone after removal of the oral bandages were performed, which may be considered a study limitation. Nevertheless, it is proposed that the neural mechanisms underlying these phenomena could differ between skin and intraoral mucosa.

Low-force von Frey filament stimulation activates large-diameter A-beta fibers, while small-diameter C- and A-delta fibers are activated at higher force levels (pinprick stimulation).[9] Cutaneous application of capsaicin induces an ongoing discharge in C-nociceptors in the primary zone. In the secondary zone, not only nociceptive stimuli but also normally innocuous tactile stimuli become capable of producing pain mediated by A- beta mechanoreceptors.[9,12] In the present study, the capsaicin induced differential desensitization effects between low-force von Frey stimulation and high-force von Frey or pinprick stimulations. This may be explained by the fact that the tactile and noxious stimulations activate different sets of receptors.[28]

Application of Menthol

Menthol can induce a decreased cold pain threshold, mechanical pain threshold, and mechanical pinprick hyperalgesia on hairy skin.[7] The present study did not find any significant changes in mechanical sensitivity at the test site corresponding to the primary zone or surrounding areas. One possible explanation is that the applied concentration of menthol was too low to induce a robust pain sensation and subsequent somatosensory changes. However, the present study used the same concentration that has been reported to produce significant pain and distinct somatosensory changes when applied to the skin.[6,29] The cutaneous hypersensitivity to nociceptive mechanical stimuli after menthol application can be explained by the activation of TRPM8 (transient receptor potential cation channel, subfamily M, member 8) receptors, which are located on C-fibers and cold-specific A-delta fibers mediating pinprick stimuli.[30] The present findings strongly suggest significant differences between cutaneous and intraoral sensitivity to menthol application that may be due to variations in TRPM8 receptor density or differences in biophysical properties of the skin and oral mucosa.

Application of Isotonic Saline

The present study revealed an unexpected desensitizing effect of 512-mN stimuli after saline application. The decreased sensitivity to mechanical stimuli was located in the secondary zone, while no significant somatosensory change occurred in the primary zone. Therefore, it could be argued that the desensitization was nonspecific, ie, not caused by the applied substance, but instead it could possibly be explained by a more general adaptation to mechanical stimuli or to other factors such as the presence of a template or the application or removal of the oral bandage.[23] This unexpected finding stresses the importance of an appropriate control condition when somatosensory sensitivity is tested.

COG Coordinates

The COG represents the position of the center of weighted average location of the NRS scores or PiPT values. The COG results indicate that the region anterior and above (away from teeth) is more sensitive than the region posterior and below (close to teeth). This is in accordance with the results of a previous study (in which no substances were applied to the gingiva)[15] and may reflect the fact that the epithelium varies markedly in thickness and degree of keratinization and hydration as well as in nerve-fiber density across the different intraoral regions. Comparison of COG coordinates over time indicated a consistency in somatosensory sensitivity location.

Conclusions

The spatial effects caused by the application of capsaicin and menthol to the gingiva appear to differ from those obtained by the application of the same substances to the skin. Intraoral capsaicin induced robust mechanical desensitization in the primary zone, while menthol did not induce any significant changes to mechanical stimuli. The different mechanisms of intraoral somatosensory changes after induction of experimental pain need to be investigated further, but the present study clearly demonstrates that it is possible to assess variations in the intraoral sensitivity caused by a variety of mechanical stimuli. This may be important for examination of intraoral pain sensitivity in, for example, atypical odontalgia, traumatic trigeminal neuropathic pain, and burning mouth syndrome.

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