Region-Specific Effects of Trigeminal Capsaicin Stimulation

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Submitted July 12, 2018;
accepted October 31, 2018.

Aims: To investigate the region-specific effects of painful trigeminal capsaicin stimulation in healthy participants. **Methods:** Twenty healthy participants (10 men and 10 women) participated in four sessions in which they received application of 0.05 mL Vaseline (placebo) or capsaicin cream (0.1%) to a different area innervated by the three branches of the trigeminal nerve: the supraorbital area (V1), the nasal mucosa (V1/V2), and the maxillary (V2) and mandibular (V3) oral mucosa. The participants rated their perceived sensations on a 0–50–100 numeric rating scale (NRS). Thermal (5°C, 23°C, and 50°C) and mechanical (32 mN and 256 mN) sensitivities were assessed. The Schirmer tearing test was used to monitor the lacrimation level as a local measure of autonomic activity, and the Task Force Monitor was used to record systemic autonomic activity. Data were analyzed using repeated measures analysis of variance. **Results:** Capsaicin application evoked significantly higher overall NRS scores ($P < .001$) and induced significantly higher ratings to the heat stimuli ($P < .009$) in all sessions compared to control. For lacrimation level, capsaicin stimulation resulted in a significant increase compared to control ($P < .0002$) only in the nasal mucosa session. **Conclusion:** Topical application of capsaicin cream to the different branches of the trigeminal nerve caused higher NRS scores along with an altered somatosensory sensitivity. Furthermore, in the nasal mucosa session, a robust local and generalized parasympathetic activation appeared following capsaicin application. *J Oral Facial Pain Headache 2019;33:318–330. doi: 10.11607/ofph.2303*

Keywords: *autonomic nervous system, capsaicin, experimental pain model, trigeminal nociception, trigeminal parasympathetic reflex*

Headaches and orofacial pains represent an array of heterogenous painful conditions. However, these conditions share physiologic activity that involves one or more branches of the trigeminal nerve, which has three major divisions: the ophthalmic division (V1), the maxillary division (V2), and the mandibular division (V3).

Trigeminal autonomic cephalalgias (TACs) are distinct subtypes of primary headaches in which the autonomic nervous system is strongly involved.[1] During TAC attacks, a minimum of one out of the following clinical features are expressed: lacrimation, nasal congestion, conjunctival injection, rhinorrhea, ptosis, and/or miosis.[1] These symptoms suggest a combination of parasympathetic activation and sympathetic inhibition. Cluster headache (CH) is the most common form of TAC, with a prevalence of about 0.1% and a male predominance.[2] Current pathophysiologic theories suggest that the trigeminovascular system, the parasympathetic nerve fibers, and the hypothalamus all play a key role in CH. However, more studies are needed to fully understand the complex interaction among the trigeminal distribution of pain, the accompanying autonomic responses, and the circadian and circannual patterns of attacks.[3]

In order to improve the knowledge of the nociceptive processing and symptomatology of pain conditions such as CH, human experimental pain models must be applied.[4,5] One such model involves brief exposure of ammonium to the nasal mucosa, which evokes a localized and intense painful sensation associated with ipsilateral lacrimation.[5]

Capsaicin application is also considered a valid experimental pain model.[6] Capsaicin cream has been tested extensively on the skin[7–11] and on the oral mucosa,[12–17] but, to the present authors' knowledge, it has not been applied to the nasal mucosa. Capsaicin applied to the oral mucosa evokes moderate to intense levels of pain associated with changes in somatosensory sensitivity,[14,15,17,18] but no systematic reports of activation of the autonomic nervous system have been described. Frese et al (2003) performed subcutaneous capsaicin injections into the V1 area of healthy individuals and observed high-intensity pain and robust local autonomic activation with ipsilateral lacrimation and miosis.[4] In the same study, Frese et al mentioned that according to observations from earlier studies, a similar reaction was not seen when injecting the V3 area. However, no data on this were presented. Thus, there is no evidence to substantiate whether peripheral painful stimulation of the V1 and V3 regions causes different or the same autonomic activation. Autonomic symptoms during CH attacks are seen in more than 90% of patient cases,[19,20] whereas autonomic features in neurovascular orofacial pain (NVOP) attacks located in the V2 and V3 regions are much less prevalent, occurring in 10% to 30% of patients.[21,22] This observation raises the question as to whether there is a difference in nociceptive sensitivity and autonomic nervous connectivity between the different divisions of the trigeminal nerve.

The overall aim of the present study was to investigate the region-specific effects of capsaicin stimulation of different orofacial tissues innervated by the three divisions of the trigeminal nerve. The following was hypothesized: *(1)* Capsaicin application provokes pain and alters somatosensory function, which differs between the three branches of the trigeminal nerve; and *(2)* capsaicin stimulation of areas innervated by V1 triggers the trigeminal-parasympathetic reflex, thereby activating the autonomic nervous system.

Materials and Methods

Participants

Twenty healthy participants (10 men and 10 women, mean age ± standard deviation [SD] 25.0 ± 4.0 years) were recruited through advertising at Aarhus University campus and at the webpage: www.forsoegsperson.dk.

Participants were provided with both written and verbal information regarding the study and prior to enrollment signed an informed consent. The inclusion criteria were good health; aged between 18 and 40 years; intact skin and mucosa on the application areas; and no orofacial pain complaints or recurrent headaches within the last 6 months. The exclusion criteria were allergy to capsaicin; an abnormal electrocardiogram (ECG); pharmacologic treatment affecting the cardiovascular or autonomic nervous system; diabetes mellitus; infection with human immunodeficiency virus; Raynaud syndrome; hypertension; depression; and drug abuse. Before the final enrollment, participants underwent a 12-lead ECG screening to ensure that no abnormalities unfamiliar to the participant were present. A medical doctor (A.J.T.) verified the ECG screenings. Prior to each session, participants were told to meet the following criteria: No intake of alcohol and beverages or food containing caffeine, as well as abstaining from excessive physical activity, for at least 12 hours before an experimental session. The participants were also required to fast for a minimum of 2 hours prior to each session.

Study Design

The study was conducted in accordance with the guidelines of the World Medical Association Declaration of Helsinki and was approved by the Central Denmark Region Committees on Biomedical Research Ethics (No. 1-10-72-57-17). The study was performed in a randomized, single-blinded, placebo-controlled, crossover manner (Fig 1). The full procedure included four sessions with a minimum of 7 days between each session to minimize the risk of carryover effects. Each session started with 20 minutes of acclimatization, followed by the same order of sequences: baseline 1; Vaseline application; baseline 2; capsaicin application. The order of the four sessions (supraorbital region, nasal mucosa, maxillary mucosa, or mandibular mucosa), as well as the side of application (left or right), were randomized. An experimental session lasted 75 minutes and was performed in a quiet room with the temperature set at approximately 23°C. The participants rested on a dental chair in a supine position during the entire session. The same examiner (C.E.P.) performed all the clinical procedures. The stimulation areas were the skin 1 cm superior to the middle part of the eyebrow, the nasal mucosa covering the septal cartilage (5 mm from the entrance of the nostril), and the oral mucosa adjacent to the first premolar in the maxilla and in the mandible.

Conditioning Stimuli

Capsaicin was prepared at a 0.1% concentration (Capzasin-HP, Chattem),[14,15,18] and a syringe was used to dosage the amount of 0.05 mL. Vaseline (Apotekets Vaseline) was used as the placebo control. The cream was applied for a duration of 7 minutes, and afterwards it was gently wiped off with a cotton swab in order to avoid a slick surface of the test area (Fig 1).

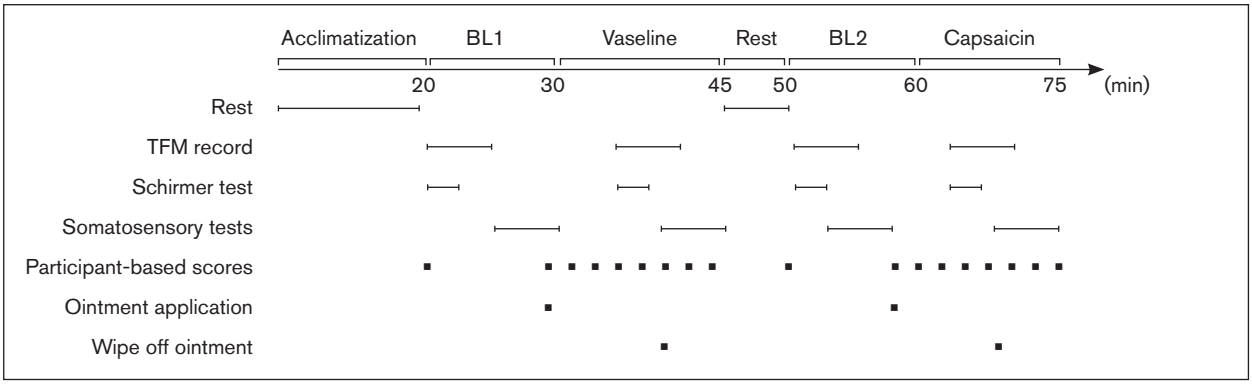

Fig 1 Illustration of an experimental session. Participants acclimatized for 20 minutes before baseline 1 (BL1) registrations were initiated: participant-based scores, 5 minutes of Task Force Monitor (TFM) recording, 2 minutes of the Schirmer test, and 5 minutes of somatosensory testing. Next, Vaseline (placebo control) was applied, and the participant-based scores were attained immediately after application and every second minute for 14 minutes. Apart from the additional participant-based scores, registrations were a repetition of those performed during BL1. Following Vaseline stimulation, participants rested for 5 minutes to acclimatize. Registrations in the second half of the experimental session—baseline 2 (BL2) and capsaicin stimulation—were identical to those made in the first part of the session.

Participant-Based Scores

Immediately after the application and every second minute for 14 minutes, the participants rated their perceived sensations from the stimulus on a 0 to 50 to 100 numeric rating scale (NRS). Thorough and standardized instructions were given for how to use the NRS, on which 0 was defined as "no sensation at all," 50 was defined as "the first sensation of pain," and 100 was defined as "the most pain imaginable."[17,23] Thus, the scale provided information on both nonpainful sensations of increasing intensity from 0 to 49 and painful sensations of increasing intensity from 50 to 100 (Fig 1).

Somatosensory Function

The assessment of somatosensory function consisted of measuring both thermal and mechanical sensitivities. The somatosensory tests were performed at baseline 1 and baseline 2, as well as 7 minutes after the application of both capsaicin and placebo, when the peak pain was estimated to strike based on the results of the pilot study performed prior to this study. The somatosensory tests were performed in the same sequence, first thermal and then mechanical. During the intraoral sessions, a lip retractor (dental adult size double-headed T-shape intraoral cheek lip retractor opener, Zenith-Dental) was used to avoid any contact with the cheek or lip.[24]

Thermal. Thermal sensitivity testing was performed with the use of a custom-made thermal aluminum cylinder device with a blunt 5-mm–diameter contact area.[12,24] Three different temperatures of the thermal stimuli—heat (50°C), room temperature (23°C), and cold (5°C)—were applied. A warm water bath (50°C) (Salvis WB4ST) was used to preheat the thermal devices for the heat stimuli, and a cold water bath (5°C) was used to precool the thermal devices for the cold stimuli. The room-temperature cylinders were already adapted to room temperature, as they were stored in the laboratory where the experimental procedures took place. The three different temperatures of the thermal stimuli were applied three times each in a randomized order, and a mean value score for each temperature stimulus was calculated. In order to avoid temperature changes during the stimulation, a total of seven thermal devices were used (three for cold, three for warm, and one for room temperature), so that each thermal device with a temperature different from room temperature only had to be used once. The aluminum cylinders were quickly dried with a towel after being removed from the water baths just before application. All thermal stimulations were applied for 2 seconds on the application area and were performed gently to avoid any uncomfortable pressure sensations. The participants were given 5 seconds to rate the perceived intensity of the stimulus on the NRS. Special care was taken to stimulate only the application area so that unwanted perceptions of the surrounding areas were avoided.

Mechanical. Mechanical sensitivity testing was performed using calibrated von Frey nylon filaments (OptiHair, MARSTOCKnervtest).[25,26] The method of levels[27] was used, in which two specific stimulus intensities were delivered (32 mN and 256 mN). The 32-mN filament was used to provoke a tactile (nonpainful) response, and the 256-mN filament was used to provoke a noxious (painful) response.[14] Like for the thermal tests, the participants were stimulated for 2 seconds on the application area and were asked to rate the sensation on the same NRS.[25] The two mechanical stimuli were applied three times each in a randomized order, and a mean value score was calculated for each of the two different stimulus intensities.

Autonomic Nervous System Parameters

Local Activity of the Autonomic Nervous System. In order to assess tearing level, the Schirmer tearing test (Schirmer-Plus, DINA-HITEX) was applied. The Schirmer test filter paper was carefully and gently placed inside the lower eyelids of both eyes and left there for 2 minutes[28] while the participants were asked to close their eyes and relax their eyelids.[29] The amount of moisture absorbed by the filter paper (in millimeters) from each eye provided an objective measure of the lacrimation level.[30]

Systemic Activity of the Autonomic Nervous System. The Task Force Monitor (TFM; CNSystems Medizintechnik AG) noninvasively and continuously recorded the ECG, beat-to-beat blood pressure, impedance cardiography, and respiration (RESP) throughout the entire four sessions. Sequences of 5-minute recordings were extracted for data analyses.[31] From these data, mean values of the following parameters were estimated: heart rate variability (HRV) in the time (milliseconds) and frequency domains; systolic and diastolic blood pressures (sBP/dBP; mmHg); stroke volume (SV; mL); cardiac output (CO; L/min); total peripheral resistance (TPR; dyne*s/cm^5); RESP (breaths/minute); and baroreceptor sensitivity (BRS; ms/mmHg). For further details, see Terkelsen et al.[32]

In order to assess the HRV in the time and frequency domains, raw data from an ECG lead II were used. A custom-made software (Aalborg University) was used to manually investigate all ECG recordings in order to validate their correctness and remove false detections due to noise and arrhythmias. This was done by a biomedical engineer (J.H.). Each of the 20 participants had four sessions that each contained four ECG recordings, giving a total of 320 sequences. In 33 of these, corrections of ectopic R peaks and missing heartbeats were replaced. HRV in the time domain was given as the mean of all normal RR intervals (mean RR interval; ms), SD of all normal RR intervals (SDNN; ms), and the square root of the mean-squared differences of successive normal RR intervals (RMSSD; ms).[31] HRV in the frequency domain was given as low-frequency power (LF-power; ms^2/Hz), coefficient of LF component variance (CCV-LF; %), high-frequency power (HF-power; ms^2/Hz), coefficient of HF component variance (CCV-HF; %), and total power (ms^2/Hz). For power spectral analysis, an autoregressive method was used, with a model order of 24.

Statistical Analyses

The results are presented as mean ± SD. Prior to the analyses, the outcome parameters were evaluated using Q-Q plots. To accommodate the assumption of a normal distribution, the SDNN and RMSSD, as well as the HRV measures in the frequency domain, were log transformed before analysis. The remaining parameters were all normally distributed. The NRS scores for the perceived sensations were analyzed using three-way repeated measures analysis of variance (ANOVA) between session (supraorbital, nasal mucosa, maxillary mucosa, and mandibular mucosa) and stimulus (capsaicin and placebo/control) at the different time points (baseline, immediately after stimulus, and every second minute for 14 minutes). The NRS scores for the thermal and the mechanical tests were also analyzed using three-way repeated measures ANOVA between session, stimulus (temperature [5°C, 23°C, and 50°C] or pressure [32 mN and 256 mN]), and time as factors. The Schirmer tearing tests were analyzed using four-way repeated measures ANOVA with the factors session, stimulus, side (cream application side and control side), and time (baseline and time of stimulus). The HRV measurements and the hemodynamic parameters were compared using three-way repeated measures ANOVA, with the factors session, stimulus, and time (baseline and time of stimulus). When relevant, the Tukey honest significant difference (HSD) test was performed. The level of significance was set at $P < .05$.

Results

All participants completed the study. There were no side effects reported or observed.

Participant-Based Scores

The overall main-effects analysis showed that NRS scores differed significantly regarding session (ANOVA: F = 23.5; $P < .001$), stimulus (ANOVA: F = 227.5; $P < .001$), and time (ANOVA: F = 96.3; $P = 0$). The mean NRS scores following both capsaicin and placebo application in the four different sessions are illustrated in Fig 2. In all sessions, at all time points different from baseline and at 0 minutes after application, NRS scores were higher for capsaicin compared to placebo (Tukey: $P < .001$). Also, post hoc tests demonstrated significantly higher NRS scores when comparing the application of capsaicin on the nasal mucosa to the other stimulation areas at all times different from baseline and at 0 minutes after application (Tukey: $P < .001$).

Somatosensory Tests

Thermal Test. There were significant overall main effects on the NRS ratings to the cold temperature stimulus (5°C) in regard to session (ANOVA: F = 17.7; $P < .0001$), stimulus (ANOVA: F = 8.5; $P < .02$), and time (ANOVA: F = 35.0; $P < .0002$) (Fig 3). In the supraorbital region and the nasal mucosa, NRS ratings

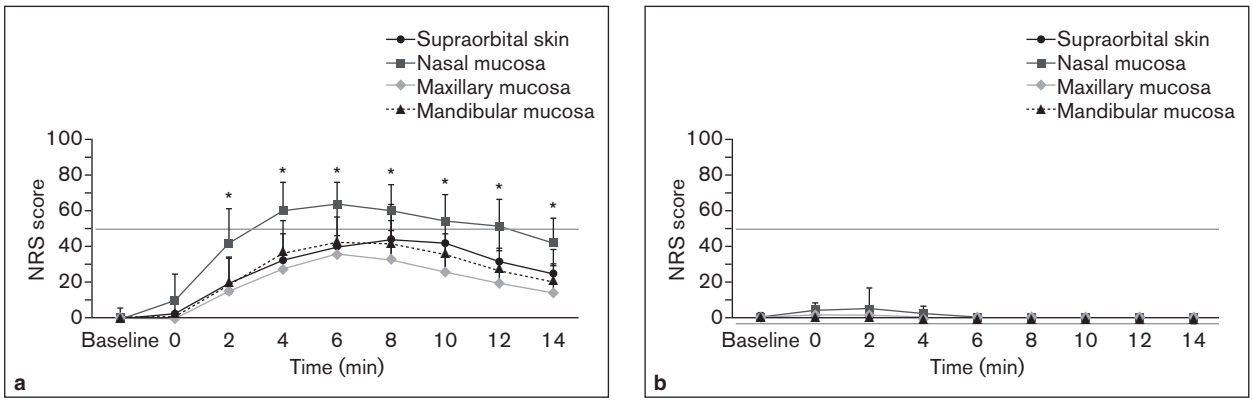

Fig 2 Participant-based scores (mean ± standard deviation) on a 0–50–100 numeric rating scale (NRS). **(a)** Ratings following capsaicin stimulation at baseline, immediately after, and every 2 minutes for 14 minutes after application. Capsaicin was perceived as painful only in the nasal mucosa (NRS > 50). In all sessions, at all time points different from baseline and at 0 minutes after application, NRS ratings were higher for capsaicin compared to placebo control (Tukey: $P < .001$). **(b)** Ratings following Vaseline stimulation at baseline, immediately after, and every 2 minutes for 14 minutes after application. *$P < .05$.

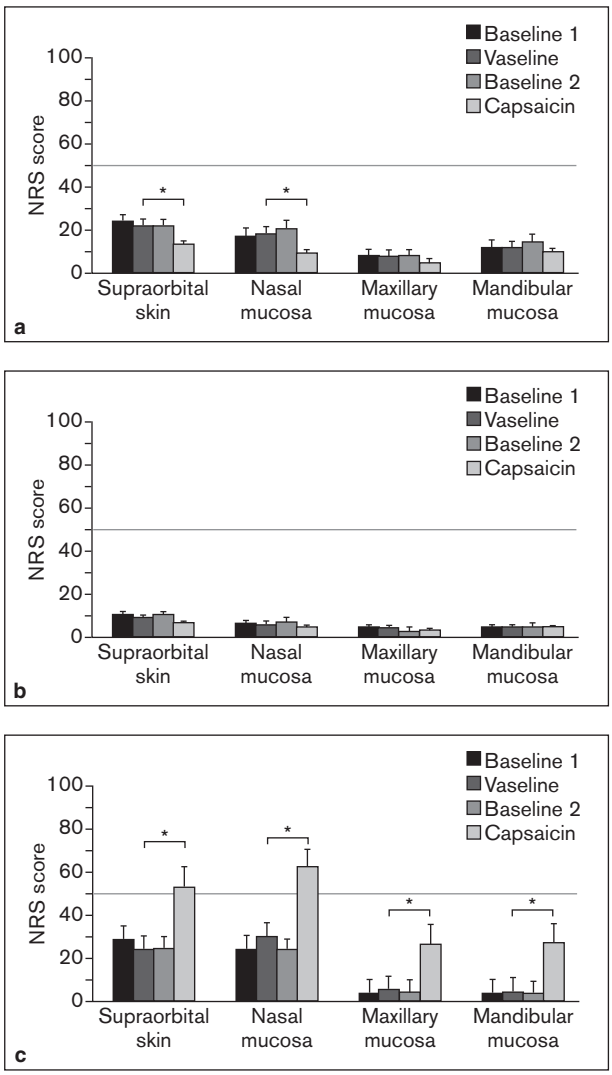

Fig 3 Mean thermal sensitivity scores (± standard deviation) on a 0–50–100 numeric rating scale (NRS). **(a)** Cold stimulus (5°C). **(b)** Room-temperature stimulus (23°C). **(c)** Warm stimulus (50°C). *$P < .05$.

decreased significantly during capsaicin stimulation compared to control (Tukey: $P < .003$). Analysis of overall main effects showed that NRS ratings to room temperature stimulus (23°C) were significantly different regarding session (ANOVA: F = 15.7; $P < .001$), stimulus (ANOVA: F = 6.39; $P < .05$), and time (ANOVA: F = 5.32; $P < .05$); the only significant finding with the post hoc tests was a decrease in NRS ratings when stimulating with capsaicin compared to control in the supraorbital session (Tukey: $P < .006$). There were significant overall main effects on the NRS ratings for the warm temperature stimulus (50°C) concerning session (ANOVA: F = 50.7; $P < .0001$), stimulus (ANOVA: F = 18.8; $P < .003$), and time (ANOVA: F = 39.8; $P < .0003$). In all sessions, a significant increase in NRS ratings was seen for capsaicin stimulation compared to control (Tukey: $P < .009$).

Mechanical Test

For the 32-mN von Frey tests, significant main effects were found regarding session (ANOVA: F = 13.8; $P < .0001$), stimulus (ANOVA: F = 5.65; $P < .04$), and time (ANOVA: F = 8.26; $P < .012$) (Fig 4). However, no significant differences in NRS ratings were seen when comparing capsaicin to control in any of the sessions (Tukey: $P > .30$).

For the 256-mN von Frey tests, main-effects analyses found significant differences in NRS ratings for session (ANOVA: F = 24.9; $P < .0001$), stimulus (ANOVA: F = 22.2; $P < .0004$), and time (ANOVA: F = 144.5; $P < .0001$). NRS ratings decreased in the supraorbital and nasal mucosa sessions during capsaicin stimulation compared to placebo (Tukey: $P = .0001$), but no significant differences in this regard were found in the sessions investigating the maxillary and mandibular mucosa (Tukey: $P > .97$).

Autonomic Nervous System Parameters

Local Activation of the Autonomic Nervous System. Main-effects analyses showed a significant difference in tearing level regarding side (active vs control) (ANOVA: F = 15.34; $P < .001$) (Fig 5). Post hoc tests showed significantly higher levels of lacrimation in the nasal mucosa session on the active side at the time of capsaicin stimulation compared to all other sessions at the same time point and on the active side (Tukey: $P < .0002$). Also, the nasal mucosa was the only stimulation site in which there was a significant increase in lacrimation following application of capsaicin cream compared to placebo (Tukey: $P < .0002$).

Systemic Activation of the Autonomic Nervous System. HRV Measures in the Time Domain. The main-effects analyses found significantly different mean RR intervals when examining the stimulus (ANOVA: F = 51.7; $P < .001$) and the time (ANOVA: F = 37.7; $P < .001$). Longer mean RR intervals were detected during capsaicin stimulation compared to control in all sessions (Tukey: $P < .047$) except for the nasal mucosa (Tukey: $P > .21$). The mean SDNN analysis only showed a main effect of stimulus (ANOVA: F = 23.7; $P < .002$). Post hoc tests revealed a significant increase in the mean SDNN for capsaicin stimulation compared to control (Tukey: $P < .003$); however, the only session in which there was a significant increase in SDNN when comparing capsaicin to control was in the nasal mucosa (Tukey: $P < .01$). The main-effects analyses of the mean RMSSD demonstrated significantly different values when examining session (ANOVA: F = 3.46, $P < .03$), stimulus (ANOVA: F = 19.2; $P < .0004$), and time (ANOVA: F = 21.8; $P < .0002$). Post hoc tests revealed significantly higher mean RMSSD values during capsaicin stimulation compared to control in the nasal mucosa and the maxillary mucosa sessions (Tukey: $P < .009$).

HRV Measures in the Frequency Domain. In the nasal mucosa session, the LF-power, HF-power, CCV-HF power, and total power increased significantly when comparing capsaicin to placebo (Tukey: $P < .001$). No such difference was present for the CCV-LF. In the maxillary mucosa, an increase in HF-power was seen when comparing capsaicin to placebo (Tukey: $P < .05$). In the remaining sessions, no differences in regard to the power spectral analysis were observed (Tukey: $P > .2$).

Hemodynamic Parameters. When comparing capsaicin to placebo, sBP increased only in the mandibular mucosa session (Tukey: $P < .0002$), whereas dBP increased in both the nasal mucosa and mandibular mucosa sessions (Tukey: $P < .001$). CO decreased in all sessions (Tukey: $P < .009$), except for the nasal mucosa (Tukey: $P > .37$). TPR increased in all sessions (Tukey: $P < .037$), and RESP only decreased in the nasal mucosa (Tukey: $P < .022$). No differences in BRS or SV were seen in any of the sessions (Tukey: $P > .099$).

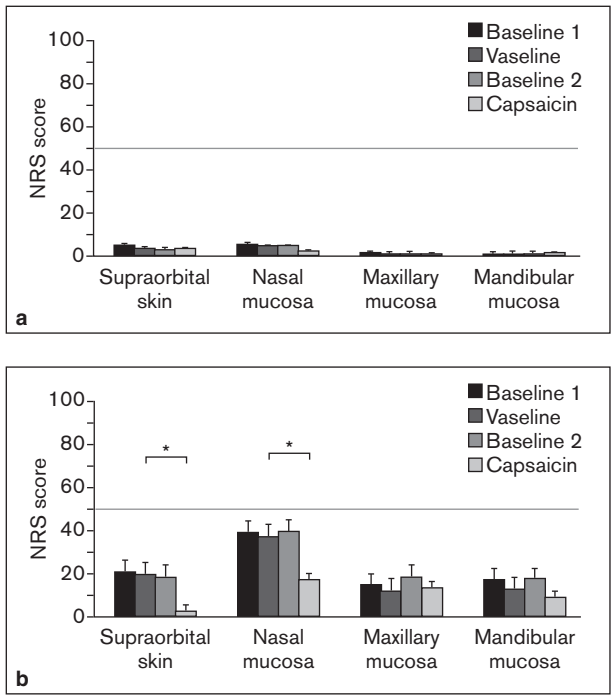

Fig 4 Mean mechanical sensitivity scores (± standard deviation) on a 0–50–100 numerical rating scale (NRS). **(a)** Light touch (32 mN). **(b)** Pinprick (256 mN). *$P < .05$.

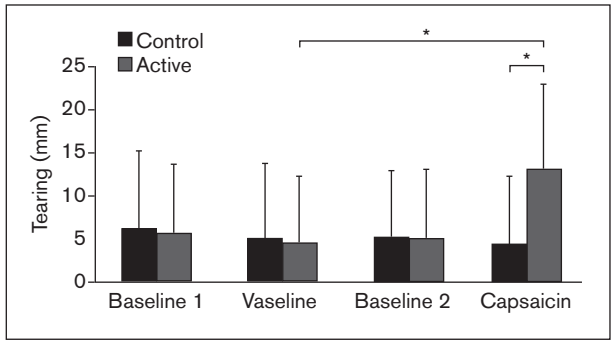

Fig 5 Schirmer tearing test in the nasal mucosa session. The Schirmer tearing test was performed bilaterally in order to have both an active side ipsilateral to the stimulation and a control side contralateral to the stimulation. The tearing test was implemented four times during an experimental session: at baseline 1, following Vaseline application, at baseline 2, and following capsaicin stimulation. The tearing level increased significantly during capsaicin stimulation when comparing the active side to the control side, as well as during Vaseline stimulation when comparing active to control. *$P < .05$.

Discussion

The main findings in this randomized, single-blinded, placebo-controlled crossover study were that capsaicin application on the nasal mucosa not only

Table 1 Heart Rate Variability (HRV) Measures in the Time Domain

	Supraorbital			
	Baseline 1	Vaseline	Baseline 2	Capsaicin
Schirmer test				
Control side (mm)	7.9 (9.6)	7.1 (8.5)	6.6 (8.4)	6.6 (8.0)
Active side (mm)	9.7 (9.3)	7.6 (8.6)	6.2 (8.1)	7.7 (9.2)
HRV measures				
Mean RR (ms)	1,007.5 (159.2)	1,037.8 (156.0)	1,049.5 (153.6)	1,066.6 (152.2)
SDNN (ms)	88.8 (43.3)	89.0 (34.3)	95.4 (38.2)	98.2 (38.7)
RMSSD (ms)	80.8 (58.1)	83.8 (49.1)	84.8 (44.3)	92.6 (49.9)
LF-power (ms^2/Hz)	247.5 (262.2)	229.8 (169.8)	230.2 (193.8)	254.5 (199.8)
HF-power (ms^2/Hz)	301.2 (396.1)	288.9 (317.8)	280.3 (268.0)	310.2 (274.3)
Total power (ms^2/Hz)	573.8 (661.7)	542.5 (466.9)	535.5 (441.4)	592.8 (466.7)
CCV-LF (%)	4.2 (2.1)	4.3 (1.7)	4.2 (1.7)	4.4 (1.8)
CCV-HF (%)	4.3 (2.7)	4.5 (2.4)	4.5 (2.2)	4.6 (2.2)
Hemodynamic parameters				
sBP (mmHg)	103.1 (10.5)	105.5 (10.7)	105.1 (9.1)	106.1 (9.2)
dBP (mmHg)	60.9 (8.6)	63.7 (8.3)	63.5 (7.3)	64.6 (6.3)
SV (mL)	107.9 (17.0)	106.4 (15.8)	105.0 (15.8)	104.8 (16.2)
CO (L/min)	6.6 (1.2)	6.3 (1.2)	6.1 (1.1)	6.0 (1.0)
TPR (dyne*s/cm^5)	944.8 (197.6)	1,019.2 (217.4)	1,043.3 (223.5)	1,076.3 (221.5)
RESP (breaths/min)	16.9 (3.2)	16.8 (3.8)	16.8 (3.4)	17.5 (2.8)
BRS (ms/mmHg)	31.8 (18.0)	32.5 (16.9)	30.8 (12.8)	33.7 (14.1)
	Maxillary mucosa			
	Baseline 1	Vaseline	Baseline 2	Capsaicin
Schirmer test				
Control side	6.8 (10.0)	5.7 (8.2)	4.5 (6.8)	4.8 (6.9)
Active side	5.6 (7.7)	5.1 (7.2)	5.8 (8.5)	5.4 (7.5)
HRV measures				
Mean RR (ms)	1,029.5 (164.5)	1,056.7 (163.0)	1,069.6 (158.3)	1,091.4 (168.0)
SDNN (ms)	88.0 (37.8)	83.4 (33.1)	91.4 (35.3)	94.1 (38.5)
RMSSD (ms)	83.3 (49.6)	83.3 (48.9)	87.9 (49.5)	95.1 (52.2)
LF-power (ms^2/Hz)	269.3 (214.2)	227.5 (167.2)	238.8 (197.6)	266.0 (196.7)
HF-power (ms^2/Hz)	287.8 (316.5)	245.9 (267.1)	292.8 (322.1)	313.2 (314.8)
Total power (ms^2/Hz)	582.2 (529.3)	496.0 (429.3)	556.6 (513.4)	606.5 (501.7)
CCV-LF (%)	4.5 (1.9)	4.1 (1.7)	4.2 (1.5)	4.4 (1.8)
CCV-HF (%)	4.5 (2.4)	4.1 (2.1)	4.4 (2.2)	4.5 (2.3)
Hemodynamic parameters				
sBP (mmHg)	103.9 (10.4)	105.4 (9.6)	104.2 (13.2)	106.1 (8.8)
dBP (mmHg)	61.9 (7.8)	63.0 (7.3)	62.9 (9.0)	65.3 (6.8)
SV (mL)	106.0 (18.0)	106.6 (18.6)	104.0 (17.7)	104.5 (17.1)
CO (L/min)	6.3 (1.3)	6.2 (1.4)	6.0 (1.2)	5.9 (1.2)
TPR (dyne*s/cm^5)	1,006.2 (255.7)	1,046.8 (270.4)	1,076.6 (287.2)	1,122.6 (260.3)
RESP (breaths/min)	16.8 (3.1)	17.7 (3.1)	17.0 (2.8)	17.6 (2.9)
BRS (ms/mmHg)	34.2 (19.9)	33.0 (12.5)	36.5 (16.5)	35.1 (15.6)

All values are reported as mean (standard deviation). Mean RR = mean of all normal RR intervals; SDNN = square root of the mean-squared differences of successive normal RR intervals (RMSSD); HRV = heart-rate variability; LF-power = low-frequency power; HF-power = high-frequency power; CCV-LF = coefficient of low-frequency power; CCV-HF = coefficient of high-frequency power; sBP = systolic blood pressure; dBP = diastolic blood pressure; SV = stroke volume; CO = cardiac output; TPR = total peripheral resistance; RESP = respiration; BRS = baroreceptor sensitivity.

caused the tearing level to increase expressively, but systemic autonomic alterations were also detected. Hopefully this study will encourage further research investigating trigeminal-parasympathetic reflexes concerning both local and systemic effects.

Conditioning Stimuli

Both cutaneous and intraoral capsaicin pain models are validated and well-described.[6,8,14–18,33] The advantage of using capsaicin as an experimental noxious stimulus is that the specific receptor mechanism shown to involve activation of the TRPV-1-receptors has been identified.[34] In this study, the amount and concentration of the capsaicin cream were based on previous studies,[18,35,36] as well as a pilot study examining intranasal application of capsaicin cream, as this has not yet previously been reported in the literature.

	Nasal mucosa			
	Baseline 1	Vaseline	Baseline 2	Capsaicin
Schirmer test				
Control side (mm)	5.9 (7.7)	4.7 (7.7)	5.2 (8.0)	13.3 (10.0)
Active side (mm)	6.3 (8.9)	5.1 (8.7)	5.2 (7.8)	4.4 (7.9)
HRV measures				
Mean RR (ms)	1,009.7 (123.7)	1,029.7 (126.0)	1,035.8 (128.7)	1,049.7 (126.1)
SDNN (ms)	93.0 (42.5)	92.1 (44.6)	95.0 (43.5)	106.1 (44.7)
RMSSD (ms)	89.8 (62.1)	88.0 (58.2)	86.1 (57.1)	99.9 (64.2)
LF-power (ms^2/Hz)	249.9 (208.0)	249.6 (274.5)	262.2 (259.3)	317.4 (220.1)
HF-power (ms^2/Hz)	330.3 (381.5)	318.2 (389.6)	297.5 (389.6)	420.7 (492.8)
Total power (ms^2/Hz)	634.0 (646.9)	631.2 (741.7)	605.1 (684.5)	769.2 (724.4)
CCV-LF (%)	4.0 (1.8)	3.7 (1.6)	3.9 (1.7)	4.6 (1.5)
CCV-HF (%)	4.0 (2.4)	3.8 (1.9)	3.5 (1.7)	4.4 (2.3)
Hemodynamic parameters				
sBP (mmHg)	103.1 (6.1)	105.3 (6.1)	105.2 (5.4)	108.9 (6.0)
dBP (mmHg)	61.4 (6.1)	63.1 (4.9)	63.2 (4.5)	66.9 (5.0)
SV (mL)	106.6 (15.6)	106.4 (15.7)	105.1 (14.7)	105.9 (17.0)
CO (L/min)	6.5 (1.3)	6.3 (1.3)	6.2 (1.3)	6.2 (1.3)
TPR (dyne*s/cm^5)	986.2 (214.6)	1,031.6 (224.7)	1,049.5 (224.1)	1,124.4 (276.0)
RESP (breaths/min)	17.6 (2.4)	17.6 (3.0)	16.8 (3.6)	16.2 (2.9)
BRS (ms/mmHg)	36.3 (21.4)	34.9 (20.1)	32.9 (16.7)	38.0 (22.0)
	Mandibular mucosa			
	Baseline 1	Vaseline	Baseline 2	Capsaicin
Schirmer test				
Control side	6.2 (7.2)	3.4 (6.6)	5.6 (7.4)	4.8 (6.7)
Active side	6.8 (9.1)	5.1 (7.7)	4.3 (6.2)	3.5 (6.5)
HRV measures				
Mean RR (ms)	988.1 (171.6)	1,009.7 (178)	1,026.7 (180.6)	1,059.8 (194.0)
SDNN (ms)	77.3 (39.5)	80.6 (36.8)	84.1 (32.9)	87.4 (38.5)
RMSSD (ms)	70.0 (50.2)	73.3 (48.1)	73.4 (45.1)	79.9 (48.4)
LF-power (ms^2/Hz)	190.5 (204.4)	182.8 (142.8)	188.2 (132.7)	210.1 (188.6)
HF-power (ms^2/Hz)	206.6 (270.9)	216.9 (260.1)	213.6 (230.6)	220.2 (225.2)
Total power (ms^2/Hz)	418.3 (468.3)	418.8 (389.7)	420.7 (345.8)	452.7 (397.0)
CCV-LF (%)	3.8 (1.6)	3.8 (1.5)	3.9 (1.3)	3.9 (1.6)
CCV-HF (%)	3.6 (2.1)	3.7 (1.9)	3.7 (1.9)	3.8 (1.9)
Hemodynamic parameters				
sBP (mmHg)	104.7 (11.1)	104.8 (9.6)	106.7 (9.7)	110.5 (10.8)
dBP (mmHg)	61.7 (8.5)	62.5 (7.5)	63.7 (7.4)	67.2 (7.5)
SV (mL)	106.4 (18.6)	105.3 (18.1)	104.4 (17.3)	103.3 (17.3)
CO (L/min)	6.6 (1.2)	6.4 (1.2)	6.3 (1.1)	6.0 (1.2)
TPR (dyne*s/cm^5)	955.6 (222.5)	992.4 (231.7)	1,039.6 (258)	1,140.7 (297.3)
RESP (breaths/min)	17.2 (3.8)	17.7 (3.4)	17.2 (3.8)	18.0 (2.7)
BRS (ms/mmHg)	30.3 (32.3)	30.3 (19.8)	29.9 (16.6)	31.0 (16.7)

Participant-Based Scores

Capsaicin evoked a painful sensation when applied to the nasal mucosa, but this was not the case when applied to the other stimulation sites. This was surprising, as the capsaicin concentration in this study was based on a pilot study in which participants perceived the 0.1% capsaicin as painful. In fact, the pilot study found that the pain ratings from the nasal mucosa session were of such intensity that an increase in concentration could potentially cause the participants to drop out. Furthermore, earlier studies have demonstrated that capsaicin cream of the exact same concentration caused pain when applied to the oral mucosa.[18,35,36] A possible explanation for the conflicting pain ratings can be attributed to the use of different pain rating scales. In this study, a 0–50–100 NRS was used that contained both nonpainful (0 to 49) and painful ratings (50 and up),

Table 2 Tukey Honest Significant Difference Test for Between-Session Differences

	Sup ×			Nas ×		Max ×
	Nas	Max	Man	Max	Man	Man
Somatosensory tests (0–50–100 NRS)						
5°C	.582	**.002**	.361	.392	1.000	.617
23°C	.997	.804	.354	.999	.963	.999
50°C	.217	**< .001**	**< .001**	**< .001**	**< .001**	.566
32 mN	.999	**.004**	**.048**	.065	.392	.999
256 mN	**< .001**	.076	.321	**.007**	**.001**	.999
Schirmer test						
Active side	**< .001**	.311	**< .001**	**< .001**	<.001	.646
Control side	.398	.786	.786	1.000	.999	1.000
HRV measures						
Mean RR (ms)	.833	.160	.999	.999	.294	**.018**
SDNN (ms)	.798	.999	.312	.175	**.001**	.926
RMSSD (ms)	.746	.999	**.037**	.990	**<.001**	**.004**
LF-power (ms^2/Hz)	.277	1.000	.848	.512	**.001**	.615
HF-power (ms^2/Hz)	.775	1.000	**< .001**	.889	**<.001**	**< .001**
Total power (ms^2/Hz)	.250	1.000	**.020**	.389	**.000**	**.009**
CCV-LF (%)[a]						
CCV-HF (%)	.911	1.000	**< .001**	.699	**< .001**	**< .001**
Hemodynamic parameters						
sBP (mmHG)	**.012**	1.000	**.005**	**.009**	**1.000**	**.004**
dBP (mmHg)	**.019**	.999	.122	.180	1.000	.591
SV (mL)	.052	1.000	.928	**.018**	**< .001**	.992
CO (L/min)	.772	.823	1.000	**.013**	.750	.841
TPR (dyne*s/cm^5)	.156	.200	**.009**	1.000	.999	.998
RESP (breaths/min)	**.052**	1.000	.987	**.019**	**.001**	.999
BRS (ms/mmmHg)[a]						

All data are reported as *P* values. Significant values are in bold. Sup = supraorbital; Nas = nasal mucosa; Max = maxillary mucosa; Man = mandibular mucosa; Cap = capsaicin; Vas = Vaseline; HRV = heart rate variability; mean RR = mean of all normal RR intervals; SDNN = standard deviation of all normal RR intervals; RMSSD = square root of the mean-squared differences of all successive normal RR intervals; LF-power = low-frequency power; HF-power = high-frequency power; CCV-LF = coefficient of low-frequency power; CCV-HF = coefficient of high-frequency power; sBP = systolic blood pressure; dBP = diastolic blood pressure; SV = stroke volume; CO = cardiac output; TPR = total peripheral resistance; RESP = respiration; BRS = baroreceptor sensitivity.

[a]No significance according to analysis of variance.

whereas other studies used a 0 to 10 visual analog scale (VAS)[18,35,36] only confined to painful scores. Perhaps participants in this study were less prone to score a sensation as painful because it can be difficult to pinpoint when exactly an unpleasant sensation becomes painful. One could argue that two separate scales should have been applied (one for pain and one for unpleasantness) in order to avoid having a definite differentiation of the two phenomena, as a painful sensation can also be unpleasant and vice versa. For future studies, in order to certainly provoke pain on the intraoral mucosa and the skin, a capsaicin concentration > 0.1% should be used.

Somatosensory Tests

Thermal. The 5°C thermal stimulus to the supraorbital region and the nasal mucosa resulted in significantly lower NRS ratings during capsaicin application compared to placebo. It seems feasible that this effect was seen because the cold stimulus equalizes or soothes the warming and burning sensations from the capsaicin. On the contrary, NRS scores for the 50°C thermal stimulus evoked higher NRS scores following capsaicin application in all sessions. The increased sensitivity to heat is in accordance with other capsaicin studies performed intraorally,[15,17,18] as well as on hairy skin.[7,33] This amplified response to warm stimuli might be caused by a peripheral sensitization of C fibers.[9,37] Surprisingly, the NRS ratings to the warm thermal stimulus were not painful until after the application of capsaicin. Normally, 50°C would be perceived as painful when performing somatosensory tests on both skin and oral mucosa in healthy individuals,[38] as the temperature is close to causing tissue damage. However, the diameter of the probe is 5 mm, so the application area does not affect a large receptive field. No altered response in NRS scores was present following the 23°C thermal stimulus when comparing capsaicin to both placebo and baseline.

Mechanical. Capsaicin did not have any effect on the NRS ratings to the 32-mN filament in any of the sessions. On the contrary, the capsaicin stimulus demonstrated a decrease in mechanical sensitivity toward the 256-mN filament in the supraorbital region and the nasal mucosa session compared to control. These findings, of mechanical desensitization to a high-force mechanical stimulus, were unexpected,

Cap × Vas			
Sup	Nas	Max	Man
.003	.002	1.000	.999
.005	.997	.999	.984
< .001	< .001	.009	**< .001**
1.000	.257	.999	1.000
< .001	< .001	1.000	.997
1.000	< .001	1.000	.920
1.000	1.000	.999	.972
.047	.170	.005	**< .001**
.585	.047	.323	.921
.437	.003	.074	**.009**
.999	.035	.993	.992
.830	.001	.045	.792
.927	.001	.278	.914
.997	.003	.314	.999
1.000	.055	1.000	**< .001**
.998	**.001**	.258	**< .001**
.876	.100	.518	.633
.009	.371	**.003**	**< .001**
.036	**< .001**	**.001**	**< .001**
.902	**.021**	1.000	1.000

as the authors anticipated a sensitizing effect of the 0.1% capsaicin cream. In general, studies have established that the effects of capsaicin (desensitization or sensitization) are dependent on the concentration, number of repeated exposures, and site of application/route of administration. Studies have demonstrated that a high single dose of capsaicin or repeated low dose of injected or topical capsaicin induced desensitization, while single lower doses caused sensitization.[8,37,39] The mechanical desensitization seen toward the 256-mN filament is consistent with studies performing intraoral application of 5% capsaicin,[15,17] as well as a study injecting 5% capsaicin into the periodontal ligament.[13] However, the opposite phenomena, mechanical allodynia and hyperalgesia, are seen when applying capsaicin to hairy skin.[7,10,11,33] A study in which 0.1% capsaicin was applied to the oral mucosa demonstrated no alteration of the mechanical sensitivity.[18] Since the capsaicin dose in this study was low and participants received only a single dose, it is difficult to explain the mechanical desensitizing effect. Perhaps the finding suggests a depletion of the primary afferent nociceptor.

Autonomic Nervous System Parameters

One of the main aims of the present study was to investigate the effects of a nociceptive stimulus on the autonomic nervous system. Since it was only possible to provoke a painful sensation when stimulating the nasal mucosa, the discussion of the autonomic parameters will primarily focus on the findings in the nasal mucosa session.

Local Activity of the Autonomic Nervous System. The Schirmer test served as a noninvasive method to monitor the level of lacrimation—and thereby indirectly the autonomic nervous system activity—on a local level. Participants reported the Schirmer test as being the most bothersome part of the experiment. When applying capsaicin to the nasal mucosa, the Schirmer test demonstrated a significant increase in lacrimation ipsilateral to the capsaicin application side, whereas no increase in the level of lacrimation was present ipsilateral to the control side in the capsaicin session nor ipsilateral to the application side of Vaseline in the control session. The underlying mechanism of the local parasympathetic activation is not completely clear. Presumably, the effect is seen because of capsaicin binding to TRPV1 receptors on peripheral nociceptive afferents of V1. Perhaps this input triggers the trigeminal-parasympathetic reflex through interaction with the trigeminal ganglion, the trigeminocervical complex, the superior salivatory nucleus, and the sphenopalatine ganglion,[40,41] causing the lacrimation level to increase. Table 1 demonstrates that between two consecutive Schirmer tests (eg, baseline 1 vs Vaseline and baseline 2 vs capsaicin on both the active side and the control side), the tearing level in the second test seems to decrease from the tearing level in the first test, indicating a tendency for the participant's eyes to dry out. However, the only times an increase in tearing between two consecutive tests were observed was when applying capsaicin to the supraorbital region and the nasal mucosa. This indicates a unique local parasympathetic activation when stimulating areas containing innervation from V1, but not areas exclusively innervated by V2 or V3.[4]

Numerous experimental pain studies have established a sex-related difference in pain perception, with greater pain intensity reported in women, including in the trigeminal region.[42–44] The literature is inconclusive regarding differences in the tearing level between men and women.[45] Additional analyses (not shown) were performed in order to assess whether any sex-related differences regarding the tearing level in this study could be detected. In general, there was a weak tendency for men to have a higher lacrimation level than women, which is in accordance with Sakomoto et al (1993)[46]; however, no systematic/consistent statistical differences could be

found. This could be due to the relatively small sample size and further studies could explore this issue in more detail.

HRV in the Time and Frequency Domains. The HRV, expressed by the SDNN and the RMSSD, increased significantly during capsaicin stimulation of the nasal mucosa. The autonomic profile changed, and the larger interval between consecutive R peaks indicated increased parasympathetic activity of the heart.[31] This was confirmed by the significant increase in HF-power and CCV-HF, which serve as indicators for the vagal activity of the heart.[31] Prior to the study, the authors did not know whether to expect a detectable difference in the autonomic nervous system or not. It was speculated that the following outcomes were possible: increase in sympathetic activity as a stress response to the painful sensation; an increase in parasympathetic activity if the trigeminal parasympathetic reflex would have a measurable systemic effect; or no change in the autonomic activity. In 1999, Schaller et al introduced the trigeminal cardiac reflex,[47] which can be triggered from all three branches of the trigeminal nerve and causes bradycardia mediated by the vagus nerve. From the HRV measurements it is clearly indicated that an increase in parasympathetic activity was present when comparing capsaicin to control. Whether this effect originates from the trigeminal parasympathetic reflex or the trigeminal cardiac reflex, or even from a combination of both, remains unclear, and further studies are needed to elucidate this.

Hemodynamic Parameters. The dBP and TPR were both significantly increased when comparing capsaicin to control. As can be seen from Tables 1 and 2, the sBP also tended to rise, but failed to gain statistical significance. The increase of TPR could be caused by a peripheral nociceptive vasoconstriction, thereby causing the dBP pressure to rise. The RESP decreased significantly when comparing capsaicin to control. An explanation for the latter could be that many participants reported a tendency to reduce respiration, as exhaling worsened the burning sensation from the capsaicin. Another way to elucidate the systemic autonomic findings altogether is the trigeminal cardiac reflex. Previous studies have found that peripheral stimulation of the anterior ethmoidal nerve in the nasal mucosa induces both sympathetically facilitated peripheral vasoconstriction and bradycardia through vagal mediation.[48–50]

Methodologic Considerations. The randomized, single-blinded, placebo-controlled, and crossover study design is a major strength of this experimental study. The sample size of 20 participants is relatively small, but the fact that the participants served as their own controls fortifies the small sample size. What might be considered as a bias in the study design is the fixed order of capsaicin cream always being applied last in each session. However, the authors recognized it as a necessary compromise, as it was unknown if the capsaicin cream would have a detectable effect on the cardiovascular system and, if so, for how long the carryover effect would last. An alternative approach could have been to only apply one out of the two creams at each session; however, that would have required the participants to take part in eight sessions instead of four. In order to reduce the experimental sessions, a paired study design could have been used. Nevertheless, participants were unaware of the order of application, and the authors determined the chosen study design to be the better option.

When applying both Vaseline and capsaicin to the oral mucosa, a lip retractor was used in order to avoid any contact with the cheek or lip and to prevent saliva contamination. Perhaps a bandage or a patch[15] would have been the ideal solution to completely avoid any saliva contact. However, the stimulation site was carefully observed during cream application, and no interaction with saliva was detected, probably due to the lip retractor as well as the participants' supine position.

As for the evaluation of the somatosensory function, the limited space of the intranasal application area made it impossible to utilize the gold standard for QST assessment, the Medoc Pathway Machine (thermotester, ATS, Medoc), a thermal testing method suggested by the German Research Network on Neuropathic Pain in regard to the standardized QST protocol.[25] As an alternative in this experiment, custom-made thermal aluminum cylinder devices were used in order to stimulate the nasal mucosa without touching the surrounding skin. The customized way of performing thermal tests was a necessary compromise, as no other thermal devices would fit intranasally. A previous study concluded that the reliability of a similar type of simple thermal device was excellent when stimulating on the cheek.[24]

An interesting factor that this study did not investigate is whether the participants consumed spicy foods on an everyday basis. Eating habits with continuous exposure to chili peppers could indeed have an influence on the participant's perception of capsaicin and lead to an adaptation or desensitization to its somatosensory effects.[51] Thus, a questionnaire regarding consumption of spicy foods would be appropriate to include in future studies.

The reliability of the Schirmer test can be discussed; however, the test is still the most commonly used clinical method to evaluate tear production.[52] Precautions were taken in order to make the reliability as good as possible: The same examiner performed the tests, participants were asked whether the test

strip in each eye felt equally irritating, the test was performed for 2 minutes with eyes shut without anesthesia,[28,29] and both sessions were scheduled at the same time of day. Thus, the Schirmer test served as the better solution for the following reasons: It is a more objective and reliable measure compared to having the examiner simply observe the eyes of the participants or having participants report wetting of their eyes themselves[53,54]; the method is cheap and easy to use compared to scanning fluorophotometry, which is considered the gold standard to measure tear production[55,56]; and the Schirmer test was not expected to detect minor differences in lacrimation, as a robust effect on lacrimation was anticipated based on results from the pilot study.

Finally, it may appear apprehensive to compare the same nociceptive stimulus to three different orofacial tissues, as the skin, nasal mucosa, and oral mucosa vary in several ways. Both skin and the two types of mucosa consist of stratified epithelium, but the underlying innervation densities as well as the permeability of the surface membranes differ.[57–59] However, it was of interest to the present project to illuminate the capsaicin cream's effect on these different orofacial tissues to provide useful information for future studies.

Conclusions

Capsaicin applied to the nasal mucosa evoked pain, caused somatosensory changes, and triggered a robust autonomic nervous system response both locally and systemically. However, capsaicin failed to induce a painful sensation when applied to the supraorbital region and the oral mucosa. Somatosensory changes following capsaicin application included thermal allodynia in all the tested orofacial tissues, and mechanical desensitization in the supraorbital region and the nasal mucosa were found. Furthermore, capsaicin stimulation of the nasal mucosa triggered a robust autonomic activation, evident from an increase in both local and systemic parasympathetic activity.

Acknowledgments

This project was supported by the Danish Council for Independent Research (No. 6120-00012A) and Midtiysk Bladfond. The authors report no conflicts of interest related to this study.

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