Site-Specific, Dose-Dependent, and Sex-Related Responses to the Experimental Pain Model Induced by Intradermal Injection of Capsaicin to the Foreheads and Forearms of Healthy Humans

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Aims: *To investigate whether trigeminal manifestations of pain, sensitization, and vasomotor responses following the intradermal injection of capsaicin to the foreheads differ from manifestations following injection of capsaicin in the forearms of healthy humans. Dose dependency and sex-related differences of the evoked responses were also studied.* ***Methods:*** *Twenty-eight healthy volunteers (14 women, 14 men) participated in 2 separate experiments:* (1) *Features of pain and vasomotor responses following intradermal injection of capsaicin (100 µg/100 µL) to the forehead and forearm were compared.* (2) *The features after intradermal injection of 2 different doses of capsaicin (50, 100 µg/100 µL) to the forehead were also studied. In both experiments the effect of sex was also investigated.* ***Results:*** *Experiment 1 showed that peak pain intensity (*F *[1,104] = 24.4,* P *< .001) and duration (*F *[1,104] = 13.3,* P *< .001) were greater in the forehead. However, the areas of visible flare (*F *[1,104] = 5.7,* P *< .05) and secondary pinprick hyperalgesia (*F *[1,104] = 155.1,* P *< .001) were significantly larger in the forearm. Experiment 2 indicated that peak pain intensity in the forehead was not affected by the capsaicin dose (*F *[1,52] = 1.6,* P *= .214), but duration of pain (*F *[1,52] = 6.0,* P *< .05) and perceived pain area (*F *[1,52] = 13.5,* P *< .001) were greater for the higher dose. The areas of visible flare (*F *[1,52] = 27.5,* P *< .001) and secondary pinprick hyperalgesia (*F *[1,52] = 65.6,* P *< .001) were also larger for the higher dose. In both experiments, women showed greater manifestations in several responses.* ***Conclusion:*** *Capsaicin-evoked sensory and vasomotor manifestations were different in the forehead and forearm. The differences are most likely due to the differences in innervation density and neurovascular activity. The capsaicin-induced effects were demonstrated to be dose-dependent and sex-related phenomena.* J OROFAC PAIN 2007;21:289–302

Key words: capsaicin, forearm, forehead, sex, trigeminal

Human experimental pain models of sensitization offer important and valuable insights into the underlying mechanisms of pain[1] and represent a link between animal research and clinical studies.[2] Compared with clinical research, such models provide greater homogeneity in subjects, standard and reproducible activation of nociceptive afferents, and lower costs in some cases.[3]

Topical application or intradermal injection of capsaicin is a well-known human experimental model of pain and peripheral and central sensitization.[4,5] Capsaicin activates the transient receptor potential ion channel of the vanilloid type 1 receptors (TRPV1)

on sensory afferents[6] and causes the release of vasoactive peptides, eg, calcitonin gene–related peptide (CGRP). It induces typical sensory and vasomotor symptoms, including intense burning pain,[7] flare reaction,[8] primary and secondary hyperalgesia,[5,9] increased blood flow,[10] and elevated skin temperature.[11] Such manifestations are commonly seen in clinical syndromes produced by inflammation or nerve injury.[12] Capsaicin is able to increase not only the excitability of spinal neurons[13] but also that of trigeminal nociceptive neurons.[14,15] Thus, such a model may also be a valuable tool to study the pathophysiology of some craniofacial pain conditions, such as masticatory muscle pain, temporomandibular joint pain, and primary headaches (eg, tension-type headache and migraine), which are among common clinical pain conditions.[16] The capsaicin model has been used in studies of trigeminal sensitization both in animals[17,18] and humans.[19–21]

Capsaicin-evoked responses may differ from 1 region of the body to another due to regional differences in the structure, receptor density, and reactivity of the neurovascular unit, which lead to different temporal patterns of pain.[22] Such site differences have been shown in areas innervated by the spinal cord.[22–25] However, only a few investigators have reported differences between sites innervated by the spinal cord and those innervated by the trigeminal nerve. For instance, Frot et al[26] showed higher pain ratings for topical capsaicin on the face than on the ankle. Thus, an aim of the present study was to explore the manifestations and characteristics of pain and vasomotor reactions following the intradermal injection of capsaicin to the foreheads of healthy humans compared to their forearms. The capsaicin injection to the forehead, which is innervated by the ophthalmic division of the trigeminal nerve,[27] may also provide valuable information about trigeminal pain and sensitization, which may underlie primary headaches.[28–31]

Since both the magnitude and duration of pain have also been shown to increase with the capsaicin dose[4,32] in areas innervated by the spinal cord in humans, dose dependency following intradermal injection of capsaicin to the forehead was also tested in the present study using 2 different concentrations of capsaicin. Furthermore, there is also general agreement that women have a lower threshold than men to most types of nociceptive stimuli.[33,34] However, since sex differences in capsaicin-evoked responses have not been as consistent,[25,26] the influence of sex on the evoked responses was also investigated.

Materials and Methods

Subjects

Twenty-eight young, healthy, right-handed volunteers participated in 2 separate experiments (experiments 1 and 2) separated by several weeks. The sample consisted of 14 women (mean age ± SD, 26.9 ± 5.2 years) and 14 men (mean age ± SD, 26.2 ± 4.1 years). All subjects were Caucasians and were solicited through advertisements at Aalborg University, Denmark.

None of the subjects had any history of peripheral vascular disease or neurologic or dermatologic disorders. All were nonsmokers. No medication was allowed during the experiments. No skin lesions were apparent at the test areas. The subjects were also instructed to abstain from application of topical lotions or creams on the test areas.

The female subjects were nonpregnant, normally menstruating women who were not taking oral contraceptives. None reported any induced or spontaneous abortions. The self-reported onset date of the last menses was recorded.

Signed written informed consent was obtained from all subjects before the first experiment. Participants were fully informed about the goals, procedures, and safety aspects of the study before giving their consent.

Design

The study protocol was approved by the local ethics committee (Counties of Nordjylland and Viborg, Denmark; VN 2005/37) and conducted in accordance with the Helsinki declaration.

All tests were carried out by the same investigator (PG) in a quiet room at 22 to 24°C. Throughout the experiment, the subject rested comfortably in a supine position on an adjustable bed with his or her forehead facing up and the forearms resting with the volar side up. The subjects were instructed to keep their eyes closed or averted from the testing sites.

The person who did the randomization and prepared the syringes did not take part in the injection and measurement procedures. The subjects and the investigator performing the injections were unaware of the content of the syringes.

Experimental sessions for women were performed during the early follicular phase of their menstrual cycle.

Assessments of pain intensity (on a visual analog scale [VAS]), duration, pain quality, pain distribution, flare, surface skin temperature, local blood flow, and the area of secondary pinprick hyperalgesia were performed at predetermined time points.

Laser Doppler scanning can be used to assess superficial blood flow,[39] whereas thermography reflects a local warming reaction depending mainly on increased blood flow in subcutaneous tissues.[40] Therefore measuring vascular changes by both techniques provides information on the vasomotor status of both superficial and deeper skin layers. Using these sophisticated recording techniques, it is possible to document the magnitude and distribution of vasoactive reflex patterns simultaneously.

Experiment 1. Experiment 1 was designed to investigate pain, central sensitization, and vasomotor manifestations following the intradermal injection of capsaicin to the forehead in comparison with a similar procedure in the forearm. It consisted of 2 sessions, each of which included 2 intradermal injections (100 μL; capsaicin 100 μg and isotonic saline 0.9 mg/mL). The 2 sessions were separated by 24 hours. To minimize the order effect, the study was designed so that half of the subjects received capsaicin at the forehead region in the first session and at the volar forearm in the second session and the rest of the volunteers underwent the procedure inversely. At each session, no 2 consecutive injections were given in the same region. The side (left or right) and the order of the injections were chosen at random.

Experiment 2. Experiment 2 was designed to investigate trigeminal pain manifestations following the intradermal injection of 2 different doses of capsaicin to the forehead in a randomized, double-blind manner. It consisted of 2 sessions separated by 1 month; each session included 1 intradermal capsaicin injection. To minimize the order effect, the study was designed so that half of the subjects received capsaicin 100 μg/100 μL in the first session and capsaicin 50 μg/100 μL in the second session; the rest of the volunteers underwent the procedure inversely. The injection side (left or right) was chosen at random, and care was taken to ensure that no 2 injections were given into the same mirrored position on the forehead.

Injections

Sterile solutions of capsaicin (100 μL, 50 and 100 μg) and isotonic saline (100 μL, 0.9 mg/mL; Aalborg Hospital Pharmacy) were injected intradermally with single-use Tuberculin syringes fitted with 27-gauge disposable needles. Injections to the forehead were given about 2 cm above the eyebrow, at a distance of about 3 cm from the face vertical midline. The volar surface of the forearm from the cubitus to the wrist was divided transversely into proximal, middle, and distal thirds. The injections were given into the central part of the middle third, avoiding any veins. Prior to all injections, the skin was cleaned with alcohol.

Assessment of Pain Intensity, Distribution, and Quality

The subjects were instructed to continuously rate the pain intensity evoked by each injection on an electronic VAS. A computer sampled the VAS signals every 2 seconds. Pain intensity was recorded until the subjects indicated that they no longer felt pain. Patients gave their pain a score from 0 (no pain) to 10 (the most pain imaginable). Peak pain (the highest VAS score) and duration of pain (time to complete resolution of pain) were extracted from the VAS profiles.

In experiment 2, upon resolution of pain, the subjects were also asked to draw their perceived distribution of pain (all areas of pain) on body charts. The pain maps were then digitized (ACECAD, model D9000+digitizer) to calculate the area of perceived pain.

In experiment 2, volunteers also completed either the English version of the McGill Pain Questionnaire[35] or a validated Danish version of the McGill Pain Questionnaire[36] to assess the quality of the pain. The pain rating indices (PRI) of the sensory, affective, evaluative, and miscellaneous dimensions of pain were calculated and analyzed according to Melzack,[35] and the words chosen by > 30% of subjects were noted.

Assessment of Flare

Visible flare (the reddening of the skin around the injection site) following the injections was identified by the investigator and mapped on an acetate sheet. The area was mapped 5 minutes after the injection and calculated later by a digitizer (ACECAD D9000+digitizer).

Assessment of Surface Skin Temperature

In experiment 1, the skin temperature was assessed before and 5 minutes after each injection with an infrared camera (Thermovision, Scanner 900 SW-TE, AGEMA Infrared System). The temperature resolution of the device was 0.1°C. Thermographic images were stored on a hard drive for off-line analysis of local changes in skin temperature. Surface temperature change was extracted from the differences in pre- and postinjection time points and used for statistical analysis.

Assessment of Blood Flow

In experiment 1, skin blood flow was measured before and 5 minutes after each injection by means of a laser Doppler Imager (LDI, Moor Instruments), which is a standard real-time method measuring blood flow in very small blood vessels of the microvasculature. In laser Doppler monitoring, a low-intensity laser light signal is transmitted into the skin, and the reflected light is used to measure local blood perfusion. The Doppler-shifted signal contains information about the speed and density of moving red blood cells in a tissue region. Speed and density information is processed to yield a parameter perfusion that is proportional to blood flow.[37,38]

An area of 7.5 × 7.5 cm^2 was scanned at a distance of 30 cm from the skin. The image resolution was obtained at 118 × 70 pixels with a speed of 4 ms/pixel. Each scan lasted 44 seconds. Bandwidth was set at 250 Hz to 15 kHz. Laser goggles were used to protect the subject's eyes during forehead scanning. Blood flow change (expressed in arbitrary units) was extracted from the pre- and postinjection difference in time points and used for statistical analysis.

Assessment of Secondary Pinprick Hyperalgesia

The area of secondary pinprick hyperalgesia was assessed when the injection-induced pain had vanished (about 15 minutes after the injection). A handheld calibrated von Frey nylon monofilament (40 mm length, 0.70 mm diameter, pressure 133 g/mm^2, Somedic) was used.[12] The perimeter of the area was determined along 8 radiating linear paths at 45-degree angles 6 cm in length originating from the injection site. Stimulation was started from distant starting points toward the injection site in increments of 1 cm with a 2-second interstimulus interval until the volunteer reported increased pain sensations evoked by the von Frey monofilament (pinprick hyperalgesia). Based on the marked points, which were traced on an acetate sheet, a polygon was drawn, and the area was calculated.

Statistical Analysis

Age differences between men and women were compared with the use of the unpaired *t* test.

Experiment 1. For experiment 1, data were analyzed with a 3-way analysis of variance (3-way ANOVA or 2 × 2 × 2 ANOVA). Three factors were examined in this experiment, and each factor had 2 levels. The factors were *(1)* treatment (capsaicin or saline); *(2)* region (forehead or forearm); and *(3)* sex (male or female). Since the same subjects were used for region and treatment factors, these factors were within-subject factors (repeated-measures variables).

Experiment 2. For experiment 2, data were analyzed with a 2-way ANOVA or 2 × 2 ANOVA. Two factors were examined in this experiment, and each factor had 2 levels. The factors were: *(1)* capsaicin dose (100 μg and 50 μg) and sex (male or female). Since the same subjects were used for the levels of capsaicin dose, this factor served as the within-subject factor (repeated-measures variable).

The *F* values (with degrees of freedom) and related *P* values for each factor and interactions are given. Interaction plots were created to better illustrate the existence and pattern of the interactions. All statistical tests were carried out using Sigmastat version 3.0 (SPSS), and the level of significance was set at $P < .05$.

Results

There was no significant difference in age between the male and female subjects (unpaired *t* test: $P = .692$). All volunteers completed the study, and there were no side effects.

The results of experiments 1 and 2 are summarized in Tables 1 and 2, respectively.

Experiment 1

Peak Pain Intensity. Capsaicin evoked significantly higher pain intensity in the forehead than in the forearm (F [1,104] = 24.4, $P < .001$). Women had significantly higher pain intensity scores than men (F [1,104] = 6.9, $P < .05$). There was a significant interaction between treatment and sex (F [1,104] = 5.3, $P < .05$). The interaction plot in Fig 1a demonstrates the difference between the pain induced by capsaicin and that induced by isotonic saline; this difference was significantly greater for the female subjects, which demonstrates that capsaicin-evoked pain (but not isotonic saline-evoked pain) was predominantly modulated by the female sex. There was also a significant interaction between treatment and site (F [1,104] = 16.3, $P < .001$). Figure 1b shows that the difference between the pain induced by capsaicin is greater for the forehead than the forearm, which demonstrates that the capsaicin-evoked pain (but not isotonic saline-evoked pain) was mainly modulated by the forehead site.

Table 1 *F* and *P* Values After 3-way ANOVA (Experiment 1)

	F (1,104)	*P*
Peak pain intensity		
Main effects		
Site	24.4	< .001*
Sex	6.9	< .05*
Treatment	974.7	< .001*
Interactions		
Site × sex	0.04	.834
Site × treatment	16.3	< .001*
Sex × treatment	5.3	< .05*
Site × sex × treatment	0.008	.929
Duration of pain		
Main effects		
Site	13.3	< .001*
Sex	3.2	.077
Treatment	535.3	< .001*
Interactions		
Site × sex	0.1	.727
Site × treatment	6.9	< .05*
Sex × treatment	2.4	.121
Site × sex × treatment	0.2	.654
Area of visible flare		
Main effects		
Site	5.7	< .05*
Sex	19.3	< .001*
Treatment	575.6	< .001*
Interactions		
Site × sex	0.009	.925
Site × treatment	5.7	< .05*
Sex × treatment	19.1	< .001*
Site × sex × treatment	0.008	.929
Temperature		
Main effects		
Site	37.2	< .001*
Sex	0.7	.407
Treatment	510.0	< .001*
Interactions		
Site × sex	0.1	.811
Site × treatment	27.8	< .001*
Sex × treatment	0.2	.637
Site × sex × treatment	0.02	.888
Blood flow		
Main effects		
Site	6.4	< .05*
Sex	8.0	< .01*
Treatment	200.0	< .001*
Interactions		
Site × sex	0.1	.707
Site × treatment	6.4	< .05*
Sex × treatment	12.6	< .001*
Site × sex × treatment	0.2	.617
Area of secondary pinprick hyperalgesia		
Main effects		
Site	155.1	< .001*
Sex	4.9	< .05*
Treatment	560.3	< .001*
Interactions		
Site × sex	1.4	.244
Site × treatment	155.1	< .001*
Sex × treatment	4.9	< .05*
Site × sex × treatment	1.4	.244

* Indicates significant result.

Table 2 *F* and *P* Values After 2-way ANOVA (Experiment 2)

	F (1,52)	*P*
Main effects		
Dose	1.6	.214
Sex	3.8	.056
Interaction		
Dose × sex	0.3	.607
Duration of pain		
Main effects		
Dose	6.0	< .05*
Sex	3.8	.057
Interaction		
Dose × sex	.001	.974
Body-chart pain area		
Main effects		
Dose	13.5	< .001*
Sex	26.0	< .001*
Interaction		
Dose × sex	2.3	.133
Area of visible flare		
Main effects		
Dose	27.5	< .001*
Sex	41.2	< .001*
Interaction		
Dose × sex	0.6	.454
Area of secondary pinprick hyperalgesia		
Main effects		
Dose	65.6	< .001*
Sex	5.1	< .05*
Interaction		
Dose × sex	2.9	.095

*Indicates significant result.

Duration of Pain. Capsaicin-evoked pain lasted longer at the forehead region than at the forearm (F [1,104] = 13.3, $P < .001$). The duration of pain was not influenced by sex (F [1,104] = 3.2, $P = .077$); however, there was a significant site-by-treatment interaction (F [1,104] = 6.9, $P < .05$). Similar to the pain intensity, the capsaicin-evoked (but not isotonic saline-evoked) pain duration was mainly modulated by the forehead site.

Area of Visible Flare. Mirrored superimposed flare areas are illustrated in Fig 2a. Capsaicin induced a larger area of visible flare in the forearm than in the forehead (F [1,104] = 5.7, $P < .05$), and women showed larger capsaicin-induced flare areas compared to men (F [1,104] = 19.3, $P < .001$). There was a significant sex-by-treatment interaction (F [1,104] = 19.1, $P < .001$). The interaction plot in Fig 2b shows that the capsaicin-induced flare area was greater for women than men and that it is the female sex that predominantly interacted with the treatment levels

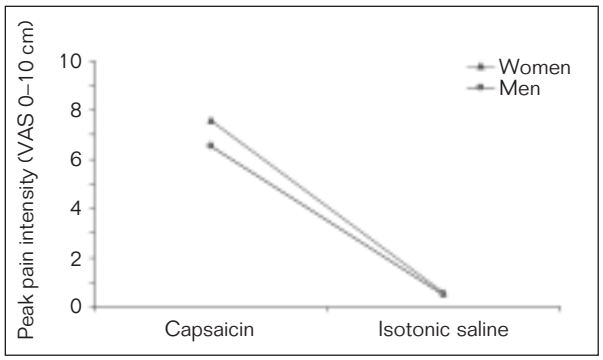

Fig 1a The treatment-by-sex interaction plot for peak pain intensity (VAS on a 0-to-10 cm scale) following intradermal injection of capsaicin (100 µg/100 µL) and isotonic saline (100 µL; 0.9 mg/mL) into the foreheads and forearms of healthy women and men.

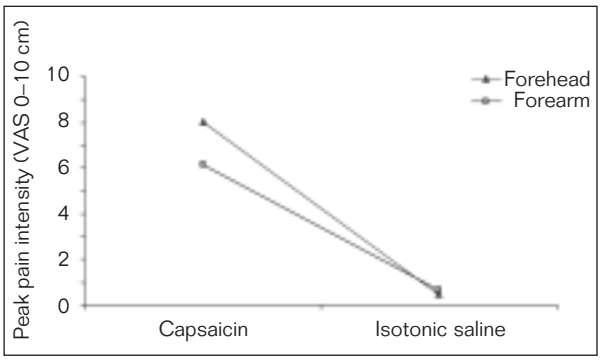

Fig 1b The treatment-by-site interaction plot for peak pain intensity (VAS on a 0-to-10 cm scale) following intradermal injection of capsaicin (100 µg/100 µL) and isotonic saline (100 µL; 0.9 mg/mL) into the foreheads and forearms of healthy women and men.

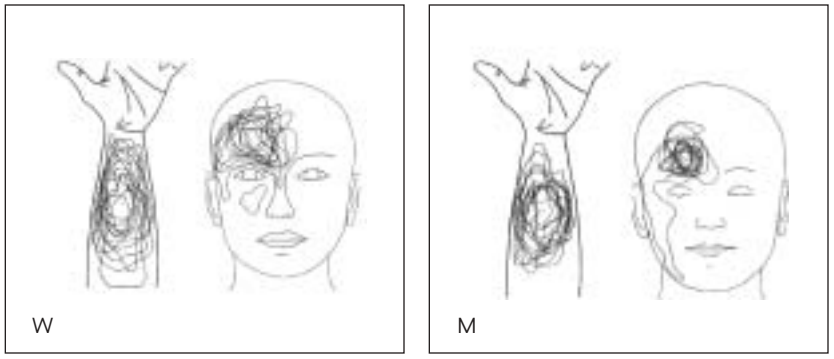

Fig 2a Flare areas superimposed on body charts following intradermal injection of capsaicin (100 µg/100 µL) into the foreheads and forearms of healthy women and men. Drawings are mirrored for half of the subjects. W = women, M = men.

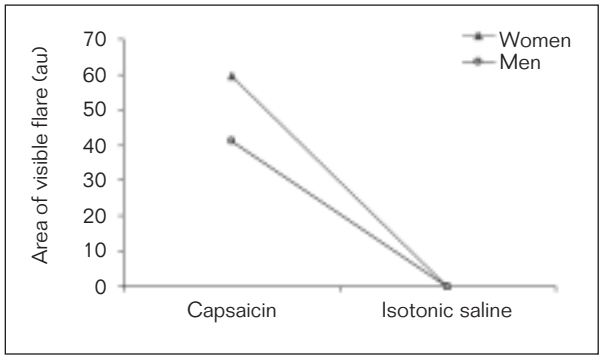

Fig 2b The treatment-by-sex interaction plot for the area of visible flare following intradermal injection of capsaicin (100 µg/100 µL) into the foreheads and forearms of healthy women and men. au = arbitrary units.

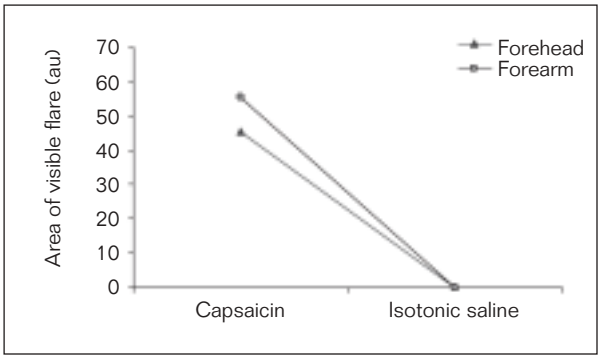

Fig 2c The treatment-by-site interaction plot for the area of visible flare following intradermal injection of capsaicin (100 µg/100 µL) into the foreheads and forearms of healthy women and men. au = arbitrary units.

(capsaicin). There was also a significant site-by-treatment interaction (F [1,104] = 5.7, $P < .05$). Figure 2c illustrates that the difference between the flare area induced by capsaicin was greater for the forearm and that capsaicin-evoked pain (but not isotonic saline-evoked pain) was mainly modulated by the forearm site.

Skin Temperature. Typical thermographic pictures of the skin temperature following the injection of capsaicin are depicted in Fig 3a. Capsaicin enhanced the skin temperature significantly more in the forehead than in the forearm (F [1,104] = 37.2, $P < .001$). This effect was not sex-dependent (F [1,104] = 0.7,

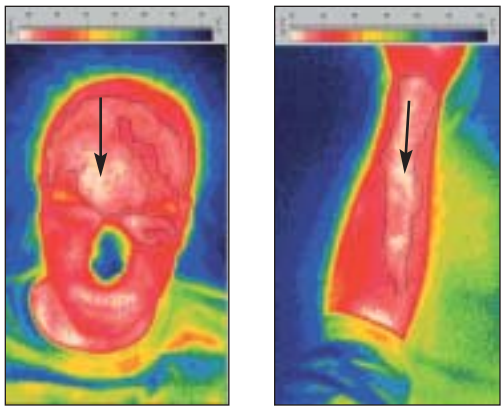

Fig 3a Typical thermographic pictures of surface skin temperature change (°C) following the injection of capsaicin (100 µL) to the forehead *(left)* and forearm *(right)* of a single male subject. The injection site is shown by an arrow, and the area with an increased temperature compared with its related baseline picture is drawn.

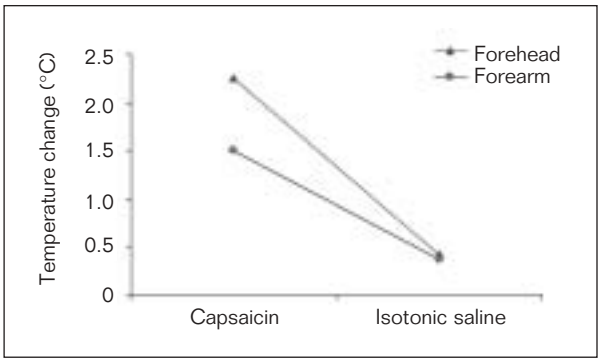

Fig 3b The treatment-by-site interaction plot for skin temperature changes (°C) following intradermal injection of capsaicin (100 µg/100 µL) and isotonic saline (100 µL; 0.9 mg/mL) into the foreheads and forearms of healthy women and men.

P = .407). There was only a site-by-treatment interaction (F [1,104] = 27.8, P < .001), and as seen in Fig 3b, the temperature change was greater for the forehead, which means that the forehead site of injection mainly modulated the capsaicin-evoked (not isotonic saline-evoked) temperature change.

Blood Flow Change. After all injections, increased blood flow was recorded. Figure 4a illustrates typical laser scanning following the injection of capsaicin. The increase in blood flow following injection was greater following the capsaicin injection compared with the isotonic saline injection, with significantly higher values for the forehead (F [1,104] = 6.4, P < .05). The change in blood flow was more pronounced in women than in men (F [1,104] = 8.0, P < .01). There were significant sex-by-treatment (F [1,104] = 12.6, P < .001) and site-by-treatment (F [1,104] = 6.4, P < .05) interactions. The interaction plot (Fig 4b) shows a greater difference between capsaicin- and isotonic saline-induced blood flow for women than for men. In fact, the female sex is the predominant factor that interacts with the treatment levels (capsaicin). Figure 4c clearly shows that the capsaicin-induced change in blood flow (but not the isotonic saline-induced change in blood flow) was mainly modulated by the forehead site.

Area of Secondary Pinprick Hyperalgesia. No subject showed any hyperalgesia following the saline injection. Capsaicin-induced secondary hyperalgesia was significantly greater in the forearm than in the forehead (F [1,104] = 155.1, P < .001), and women showed a larger hyperalgesic area than men (F [1,104] = 4.9, P < .05). There was a significant interaction between sex and treatment (F [1,104] = 4.9, P < .05). Figure 5a indicates that the female sex strongly modulated the area of capsaicin-evoked hyperalgesia.

There was also a treatment-by-site interaction (F [1,104] = 155.1, P < .001). Figure 5b shows that the site of the injection modulated the area of capsaicin-induced hyperalgesia and that the role of the forearm was greater than that of the forehead.

Experiment 2

Peak Pain Intensity. The peak pain intensity following the intradermal injection of 2 different doses of capsaicin into the forehead was not affected by dose (F [1,52] = 1.6, P = .214). There was no effect of sex on the results (F [1,52] = 3.8, P = .056). No significant interaction was observed between sex and dose (F [1,52] = 0.3, P = .607).

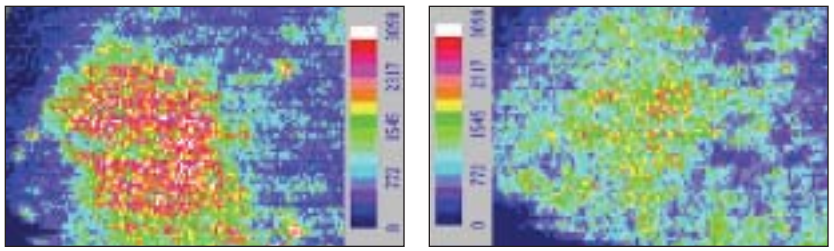

Fig 4a Typical laser scanning of skin blood flow changes following the injection of capsaicin (100 µg/0.1 mL) to the forehead *(left)* and forearm *(right)* of a subject.

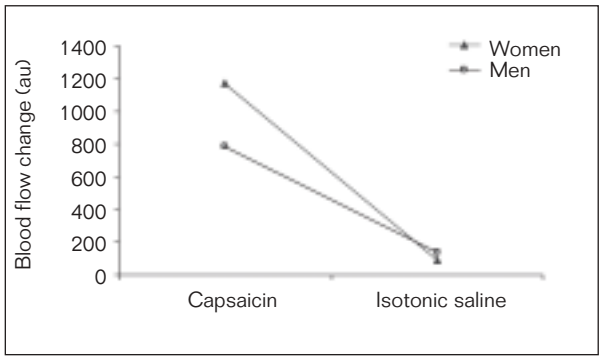

Fig 4b The treatment-by-sex interaction plot for blood flow increase as compared with baseline (au) following intradermal injection of capsaicin (100 µg/100 µL) and isotonic saline (100 µL; 0.9 mg/mL) into the foreheads and forearms of healthy women and men. au = arbitrary units.

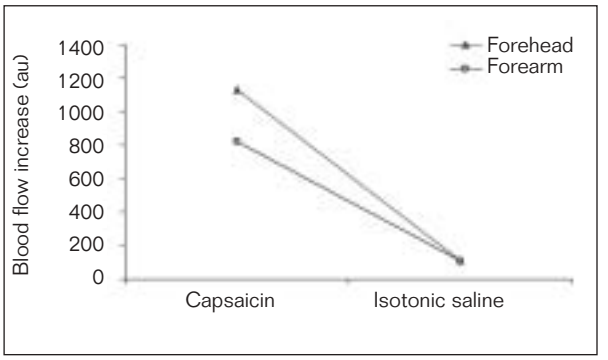

Fig 4c The treatment-by-site interaction plot for blood flow increase as compared with baseline following intradermal injection of capsaicin (100 µg/100 µL) and isotonic saline (100 µL; 0.9 mg/mL) into the foreheads and forearms of healthy women and men. au = arbitrary units.

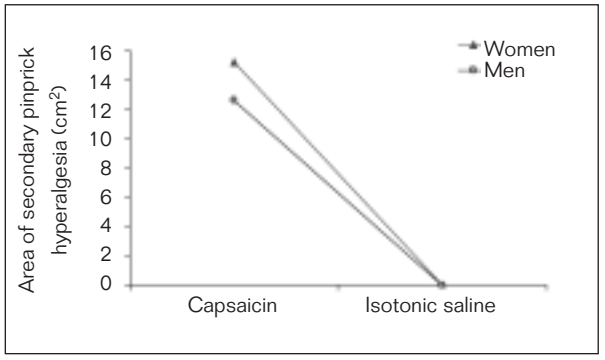

Fig 5a The treatment-by-sex interaction plot for the area of secondary pinprick hyperalgesia (cm^2) following intradermal injection of capsaicin (100 µg/100 µL) and isotonic saline into the foreheads and forearms of healthy women and men.

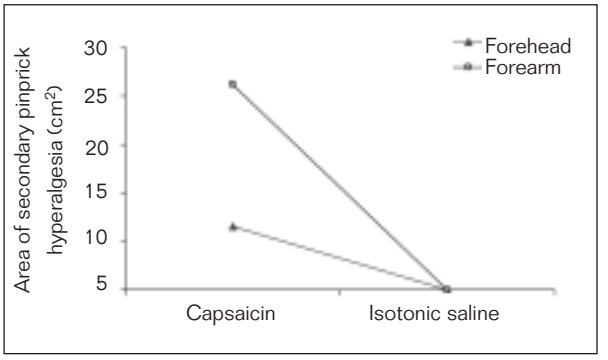

Fig 5b The treatment-by-site interaction plot for the area of secondary pinprick hyperalgesia (cm^2) following intradermal injection of capsaicin (100 µg/100 µL) and isotonic saline into the foreheads and forearms of healthy women and men.

Duration of Pain. The higher dose of capsaicin induced pain of longer duration (F [1,52] = 6.0, $P < .05$). However, the phenomenon was not affected by sex (F [1,52] = 3.8, $P = .057$). No significant interaction was observed between sex and dose (F [1,52] = 0.001, $P = .974$).

Body-chart Pain Area. Volunteers drew a larger pain area for the higher dose of capsaicin (100 µg) than for the lower dose (50 µg) (F [1,52] = 13.5, $P < .001$). Women drew a larger pain area than men (F [1,52] = 26.0, $P < .001$). Figure 6a shows the superimposed drawings. There was no significant sex-by-dose interaction (F [1,52] = 2.3, $P = .133$; Fig 6b).

Quality of Pain. Most commonly chosen words from the McGill Pain Questionnaire are given in Table 3.

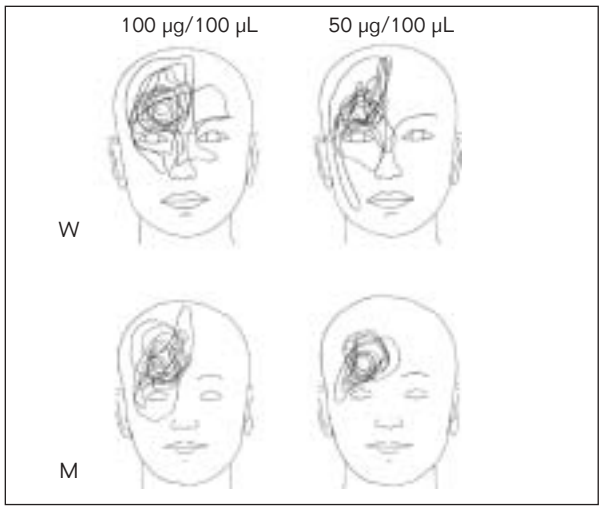

Fig 6a Superimposed face-chart pain areas following intradermal injection of capsaicin (50, 100 µg/100 µL) into the foreheads of healthy women and men. Drawings are mirrored for half of the subjects. W = women, M = men.

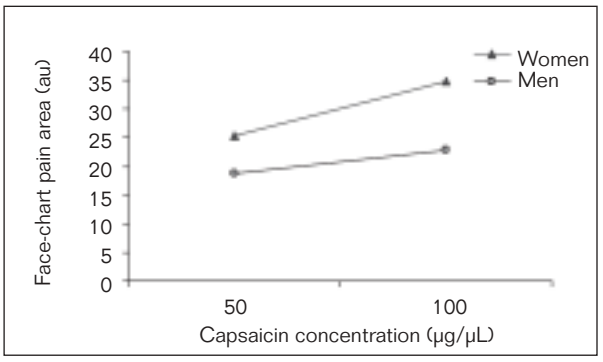

Fig 6b Face-chart pain area following intradermal injection of capsaicin (50, 100 µg/100 µL) into the foreheads of healthy women and men. au = arbitrary units.

Analysis of the PRI indices (sensory, affective, evaluative and miscellaneous) of the McGill Pain Questionnaire did not show any effect of different capsaicin doses or sex ($P > .05$). Likewise, the total number of words chosen was not affected by these factors ($P > .05$).

Area of Visible Flare. The higher dose of capsaicin (100 µg) induced a larger area of visible flare (F [1,52] = 27.5, $P < .001$). Women showed larger flare areas than men (F [1,52] = 41.2, $P < .001$). The interaction between sex and dose was not significant (F [1,52] = 0.6, $P = .454$; Fig 7).

Area of Secondary Pinprick Hyperalgesia. The capsaicin-induced secondary hyperalgesia was significantly greater with the 100-µg dose compared to the 50-µg dose (F [1,52] = 65.6, $P < .001$). The area was also larger in women than in men (F [1,52] = 5.1, $P < .05$). The interaction between sex and dose was not significant (F [1,52] = 2.9, $P = .095$; Fig 8).

Table 3 Most Commonly Chosen Words (%) from the McGill Pain Questionnaire Following Intradermal Injection of Capsaicin Into the Forehead

	Percentage (%)
Capsaicin (100 µg/100µL)	
Women	
Pressing	50.0
Burning	92.9
Stinging	42.9
Intense	50.0
Men	
Sharp	50.0
Burning	41.7
Hurting	41.7
Vicious	41.7
Intense	50.0
Capsaicin (50 µg/100 µL)	
Women	
Throbbing	42.9
Sharp	71.4
Burning	85.7
Intense	42.9
Men	
Sharp	50.0
Burning	75.0
Intense	58.3

Discussion

Site Specificity

The results of the present study have demonstrated that intradermal injection of capsaicin evoked pain of greater intensity and longer duration in the forehead than the forearm. The findings are in agreement with the results of topical application of capsaicin on the cheek, which also induced higher-intensity irritation than application on the volar forearm.[41] Higher ratings of pain and unpleasantness have also been shown after topical administration of capsaicin on the face than on the ankle.[26]

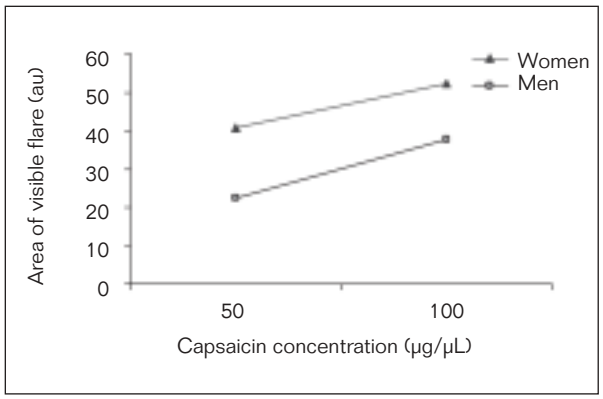

Fig 7 Area of visible flare following intradermal injection of capsaicin (50, 100 µg/100 µL) into the foreheads of healthy women and men. au = arbitrary units.

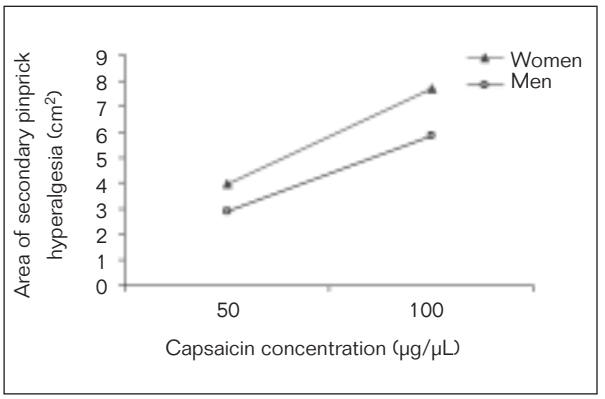

Fig 8 Area of secondary pinprick hyperalgesia (cm^2) following intradermal injection of capsaicin (50, 100 µg/100 µL) into the foreheads of healthy women and men.

Higher pain intensity in the trigeminal area compared to the spinal-innervated areas may reflect differences between these 2 systems. In both regions, sensory nerve endings express TRPV1 receptors that respond to capsaicin.[42,43] However, innervation of the orofacial regions is denser than the spinal-innervated tissues.[44] For instance, innervation density of the perioral skin is extremely high compared with spinal dermatomes.[45] It has also been shown that the number of epidermal nerves is significantly higher in facial areas (eg, upper eyelid) than in the abdomen and mammary areas.[46] In order to provide indirect evidence on innervation density in the present study, the 2-point discrimination test was performed.[47] The mean thresholds for 2-point discrimination (based on 14 subjects) were 15 mm for the forehead and 35 mm for the forearm. Thus, higher pain intensity in the forehead could be partly due to the higher innervation density of the trigeminal area. This may consequently provide a larger number of TRPV1 receptors available on the sensory nerve endings, which leads to the higher magnitude of capsaicin-evoked pain in the forehead.

Moreover, the amount and pattern of biochemical mediator release (eg, substance P) and the nociceptive neural organization in the trigeminal brainstem sensory nuclei may differ from those of the dorsal horn of the spinal cord. Such differences may also affect the magnitude of pain. Further anatomic, morphologic, and electrophysiologic studies can provide direct information on the receptor density, patterns of cutaneous innervation, and the sensitivity of sensory neurons or receptor binding properties (eg, affinity).

Flare

The results of the present study revealed that the area of visible flare was larger in the forearm; however, the magnitude of the flare in terms of blood flow and temperature was higher in the forehead. The flare response depends on the excitation of a subtype of unmyelinated C-fibers, which are called mechano-insensitive C-fibers (CMi fibers).[48–51] Unmyelinated C-fibers are more prevalent in regions innervated by the spinal cord than in those innervated by the trigeminal nerve.[44,52–54] Unmyelinated C-fibers are estimated to comprise around 15% to 25% of the C-fibers[48] and have larger, more irregular territories.[55] Thus, it is likely that the observed larger flare area in the forearm is partly due to the activation of more unmyelinated C-fibers, including CMi units, with an expanded innervation territory in the forearm. This consequently evokes neuropeptide release (eg, CGRP)[49] and provides larger axon reflex flare.

Since the flare response is related to both the neural network and vascular structure, the different distributions and sensitivities of the vascular units in addition to the type and amount of neuropeptides may also have contributed to the observed results. The vasculature pattern of the trigeminal nerve is highly complex, and it has been shown that peripheral parts of the maxillary nerve and branches of the mandibular nerve contain a great density of blood vessels.[56] Therefore, it is hypothesized that the higher vascular density or vascular reactivity of the neurovascular units could be a possible reason for the higher magnitude of flare (blood flow and temperature) in the forehead.

The pattern of autonomic nerve activation after the capsaicin injection may also differ between the

forehead and forearm. For instance, dermal blood vessels of the extremities are controlled by the sympathetic nervous system, whereas those of the head may be predominantly controlled by the parasympathetic nervous system and circulating vasoactive agents.[57]

Secondary Pinprick Hyperalgesia. The findings of the present study showed that the area of capsaicin-induced secondary pinprick hyperalgesia was also larger in the forearm. It is generally accepted that pinprick hyperalgesia is primarily mediated by A-δ fibers[58] and is due to an amplified central response (central sensitization).[59,60] The areas of secondary hyperalgesia are defined by their related central receptive fields. The central receptive fields may correlate to the peripheral receptive fields or their related dermatomes in the skin. Thus the A-δ fibers provide enlarged dermatomal segments in the forearm, which consequently leads to the existence of larger spinal receptive fields.

Sex-Related Differences

Epidemiologic studies have revealed that many painful diseases have a documented female prevalence, particularly those affecting the head and neck.[61] Women report more severe levels of pain, more frequent pain, pain in more areas of the body, and pain of longer duration than men.[62] Several experimental pain studies using different noxious stimuli have also revealed greater responses in women.[34] However, the type of stimuli, assessment methods, sex of the experimenter, and the site of induced pain may also have some influence on the sex-related responses.[33,34]

In the present study, women generally showed greater responses to the intradermal injection of capsaicin than men. The present investigators recently reported the influence of the menstrual cycle on the perception of capsaicin-induced pain and hyperalgesia.[63] An experimental pain study[26] using topical capsaicin on the face and the ankle demonstrated sex-related differences in pain perception, with greater pain intensity in female subjects, which also matches the present findings. However, in the present study, the female subjects expressed larger flare and higher increased blood flow responses than men, which contradicts the results of Ferrell and colleagues,[25] who used topical capsaicin to induce pain and vascular reactions. They found a significant sex difference in vascular responses, with greater changes in men, and a significant sex difference in temperature, with greater changes in women. The difference between the present results and their findings is presumably due to the difference in capsaicin application techniques or to female hormonal levels in both studies.

Higher pain sensitivity and greater responses of women following noxious stimuli are probably mediated by different factors, including structural and biological factors (eg, anatomic factors, morphologic factors, genetics, gonadal hormones, endogenous pain inhibition system), sociocultural factors (eg, age, ethnicity, family history, gender roles), and psychologic factors (eg, anxiety, depression, cognitive and behavioral factors). These sets of factors interact in complex ways.[64] For instance, estrogen has been demonstrated to expand the size of the receptive field area of trigeminal mechanoreceptors in the rodent.[65–67] However, plasma progesterone levels are positively correlated with the antihyperalgesia noted in behavioral studies.[68] The μ opioid receptors in the healthy female brain are also activated differently from those in the male brain.[69]

The findings of the authors' previous study[63] as well as the present study support the idea that sex is an important basic human variable that should be considered during the design and analysis of studies. It is important to consider that some painful disorders (eg, migraine) may be different in women and men and that the efficacy of some therapies may be greater in one sex than the other. Thus, considering the contribution of sex may lead to improvement of clinical diagnosis and treatment.

Dose Dependency

Data from the present study demonstrated the dose-dependent effect of capsaicin in the forehead. Pain flare and hyperalgesic areas were all larger in response to the higher dose of capsaicin. Previous human and animal studies have also demonstrated such a reaction in the trigeminal-innervated areas. In terms of pain and sensitivity, several human studies[70–72] have shown that the higher pain intensity is correlated to higher capsaicin concentration in the face or oral cavity. In an animal study by Pelissier and coworkers[17] in which different doses of capsaicin were injected subcutaneously in the orofacial regions of rats, a positive relationship was also observed between the amplitude of face-grooming activity (a behavior correlated with nociception) and the dose of capsaicin. In terms of flare, capsaicin-evoked immunoreactive CGRP release from the rat buccal mucosa, a marker of trigeminal neurogenic inflammation, has also been shown to be dependent on the concentration of capsaicin.[18]

The dose dependency of pain, flare, and hyperalgesia after intradermal injection of capsaicin into spinal-innervated areas (eg, the human forearm) has also been investigated in detail.[4,32,72–74] In the present study, a greater response to capsaicin was found with a higher dose in the forehead. Thus, the phenomenon of dose dependency is not site-dependent. The mechanism of dose dependency may be similar for the trigeminal- and spinal-innervated areas: The higher capsaicin concentration activates a larger number of the afferent nerve fibers and TRPV1 receptors, which reflects a greater magnitude of pain. Similarly, the more the fibers are stimulated, the greater the amount of vasoactive neuropeptides released and the number of neurovascular units activated and the greater the magnitude of the flare. Stronger central sensitization could also be caused by greater sensory input, which leads to stronger effects on the central nervous system.

Conclusions

The present study demonstrated sensory and vasomotor differences between trigeminal and spinal systems in response to the intradermal injection of capsaicin. The observed differences were most likely due to the differences in the sensory and autonomic innervation in terms of innervation density, neurovascular sensitivity, the pattern and magnitude of neuropeptide depletion, and functional neural organization in the trigeminal and spinal central pathways.

The present study also highlighted the capsaicin-induced effects in the trigeminal system as dose-dependent phenomena. The higher concentration of capsaicin probably recruits more neurovascular units, which subsequently leads to the stronger effect on the central nervous system.

A sex-related response to capsaicin was also indicated in the present study, with greater magnitudes of the evoked responses in women. This phenomenon is probably due to several factors, such as biological differences between men and women.

Collectively, the findings of the present study have some clinical implications:

- Pathophysiologic differences between trigeminal and spinal pain mechanisms should be taken into consideration in treatment planning for chronic trigeminal pain conditions.
- Sex is an important variable that may affect both diagnostic and treatment options of some painful disorders.

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