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Capsaicin-induced secondary hyperalgesia differences between the trigeminal and spinal innervation

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This study compared the degree of secondary hyperalgesia and somatosensory threshold changes induced by topical capsaicin between spinal and trigeminal innervation. This crossover clinical trial included 40 healthy individuals in which 0.25 g of 1% capsaicin cream was randomly applied for 45 minutes to a circular area of 2 cm^2 to the skin covering the masseter muscle and forearm in 2 different sessions, separated by at least 24 hours and no more than 72 hours (washout period). The main outcome variables were the area of allodynia and pinprick hyperalgesia, as well as electrical and mechanical pain thresholds within the area of pinprick hyperalgesia. Mixed ANOVA models and McNemar tests were applied to the data ($p = 0.050$). The occurrence of allodynia and pinprick hyperalgesia was higher in the forearm than in the masseter ($p < 0.050$). Additionally, the areas of pinprick hyperalgesia and allodynia were larger in the forearm compared to the masseter ($p < 0.050$). The electrical and mechanical pain thresholds demonstrated a loss of somatosensory function following capsaicin application to the masseter ($p < 0.050$). However, no significant somatosensory threshold changes were observed at the forearm after capsaicin ($p > 0.050$). In conclusion, these findings indicate potential differences compatible with central sensitization related to secondary hyperalgesia between trigeminal and spinal innervation.

Keywords Capsaicin, Central sensitization, Secondary hyperalgesia, Orofacial pain

The trigeminothalamic and spinothalamic neural pathways carry nociceptive and temperature information and, although they are generally considered homologous, there are anatomical and physiological differences, such as a distinct embryonic origin[1,2], a lower proportion of unmyelinated fibers relative to myelinated fibers in the branches of the trigeminal nerve[3,4] and a lower proportion of sympathetic neurons in relation to sensory neurons in the trigeminal pathway[5]. Differences in nociceptive signaling, gene expression and regulation have also been demonstrated[6]. Moreover, the expression of transient receptor potential vanilloid-1 (TRPV1) seems to be different between these regions[7]. A long isoform is found throughout the body with a thermal activation threshold of 40 °C, while a short isoform, specific to neurons innervating the head and face, has a slightly lower activation threshold[7]. Although this model is proposed, it remains subject to controversy and debate[7]. Finally, there are drugs that seem to act specifically on pain conditions affecting the trigeminal region[8,9].

It is observed that certain types of pain, such as migraine and trigeminal-autonomic headache, exclusively impact the trigeminal pathway[10]. Furthermore, the trigeminal pathway seems to be more resistant to development of post-traumatic neuropathic pain, as evidenced by the various procedures that cause injuries to the trigeminal nerve, which rarely result in orofacial neuropathic pain[11]. These findings indicate the possibility of physiological differences between trigeminal and spinal innervation, particularly in how they respond or adapt to injury.

It is also possible that the trigeminal and spinal nociceptive processing differs in terms of mechanisms and/or neural interactions in response to capsaicin. Capsaicin can induce transient somatosensory alterations

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and has been successfully applied in human investigations as a surrogate model of what has been recently conceptualized as a subtype of central sensitization where the excitability of neurons located in the spinal cord or spinal trigeminal nucleus is increased due to peripheral inputs. This subtype of central sensitization may play an important role in the onset and persistence of pain and the secondary hyperalgesia following capsaicin is a prominent manifestation that is compatible with this subtype of central sensitization[12–14]. As such, the extent and characteristics of secondary hyperalgesia may shed light on the possible physiological distinctions between the spinal and trigeminal processing of nociceptive inputs. Indeed, previous evidence suggests that secondary hyperalgesia following intradermal capsaicin application is less pronounced in the forehead than in the forearm[15].

It is also possible to assess the central processing of painful stimuli with the conditioned pain modulation (CPM). The CPM evaluates the function of various pathways of the descending pain modulatory system[16–18]. The magnitude of the inhibitory CPM response is associated with the severity of chronic pain conditions and the efficacy of pain treatments[19,20]. Moreover, a deficient inhibitory response has been considered more recently as another subtype of central sensitization[21] and with possible differences between the trigeminal and spinal regions[22]. Therefore, it is interesting to also consider the efficacy of the descending pain inhibition when assessing the secondary hyperalgesia following capsaicin application. So far, there are no studies that assessed both the CPM and the secondary hyperalgesia following capsaicin with the focus on the orofacial region.

Thus, the primary aim of this study was (1) to compare the degree of secondary hyperalgesia induced by topical capsaicin between the spinal and trigeminal innervations of healthy participants. In addition, (2) to evaluate the influence of topical capsaicin on somatosensory threshold changes in the masseter and forearm of healthy participants and (3) to explore the associations between somatosensory changes and CPM magnitude and psychosocial variables. It was hypothesized that secondary hyperalgesia would be more pronounced in the spinal than the trigeminal innervation and that sensitivity changes in response to capsaicin would differ between the two regions.

Methods

Sample and ethics

This experimental investigation included a convenience sample of 40 healthy participants that were recruited at Piracicaba Dental School, University of Campinas, Brazil. The number of male and female participants was equal in order to obtain a more representative sample of the population and reduce bias. Inclusion criteria were as follows: (a) age between 18 and 40 years (we limited the age to 40 years based on a previous study that found a prolonged duration of pinprick hyperalgesia in older adults[23]).; (b) good general health without complaints of any type of chronic pain, or episodes of orofacial pain and headache in the last 30 days, or more than 11 episodes in the last 12 months. In addition, participants were prohibited from consuming any commonly used analgesics within the 24 h preceding the sessions. The exclusion criteria were as follows: (a) presence of uncontrolled systemic disorders, e.g., diabetes, hypertension, or endocrine disorders; (b) presence of congenital or developmental diseases, e.g., aplasia, hyperplasia, dysplasia; (c) psychiatric disorders; (d) pregnancy or lactation; (e) use of medications that affect the central nervous system, such as antidepressants and anticonvulsants. A specialist in orofacial pain (I.C.N.) collected a detailed medical and dental history to determine participant eligibility.

This study was approved by the Human Research Ethics Committee of the Piracicaba Dental School (Ref: 42779821.0.0000.5418). The informed consent was obtained for each participant after a detailed explanation of the procedures and experimental design. All methods were performed in accordance with the declaration of Helsinki and the recommendation of the Human Ethics Committee. The study was not pre-registered.

Experimental design

In this crossover experimental trial, topical capsaicin 1% (*Galena Quimica e Farmaceutica Ltda*, Campinas, SP, Brazil) was applied to two different regions in two distinct experimental sessions with at least 24 h and no more than 72 h washout period between them (Fig. 1). The application sites were the skin overlying the midportion part of the volar face of the right forearm and the skin over the central region of the right masseter muscle (Fig. 1). The capsaicin cream was applied within a circular area (0.25 g within 2 cm^2), previously cleaned with alcohol, for 45 min on both the masseter and forearm, and subsequently removed. During capsaicin application, pain intensity was assessed every 5 min. The order of the sessions (cream application in one region) was randomized for each participant where half of them started with the capsaicin application on the forearm while the other half with the masseter. Men were instructed to shave the skin at the capsaicin application site the night before, rather than immediately before the application, to prevent skin irritation or small cuts that might interfere with the results.

It is important to note that an inactive nocebo cream was not utilized, as the main objective was to compare sites rather than evaluate the specific effects of capsaicin itself. Additionally, the within-subject design, coupled with a before-after assessment for each experimental session, mitigated the confounding effects of a nocebo response. Our design was informed by prior research employing the capsaicin model to investigate physiological responses, such as the temporal summation of pain[24]. Moreover, a preliminary session was conducted to familiarize individuals with the tests and to assess psychosocial functioning, as well as CPM (Fig. 1).

Outcomes

The following outcomes variables were evaluated before (baseline) and after the cream application, within the area of pinprick hyperalgesia and close to the proximal border (spinal) or posterosuperior border (trigeminal), in both the masseter and the forearm: mechanical detection threshold (MDT), mechanical pain threshold (MPT) and current perception threshold (CuPT). Baseline assessments of MDT, MPT, and CuPT were conducted in regions proximal (spinal) or posterosuperior (trigeminal) to the capsaicin application sites, targeting areas as

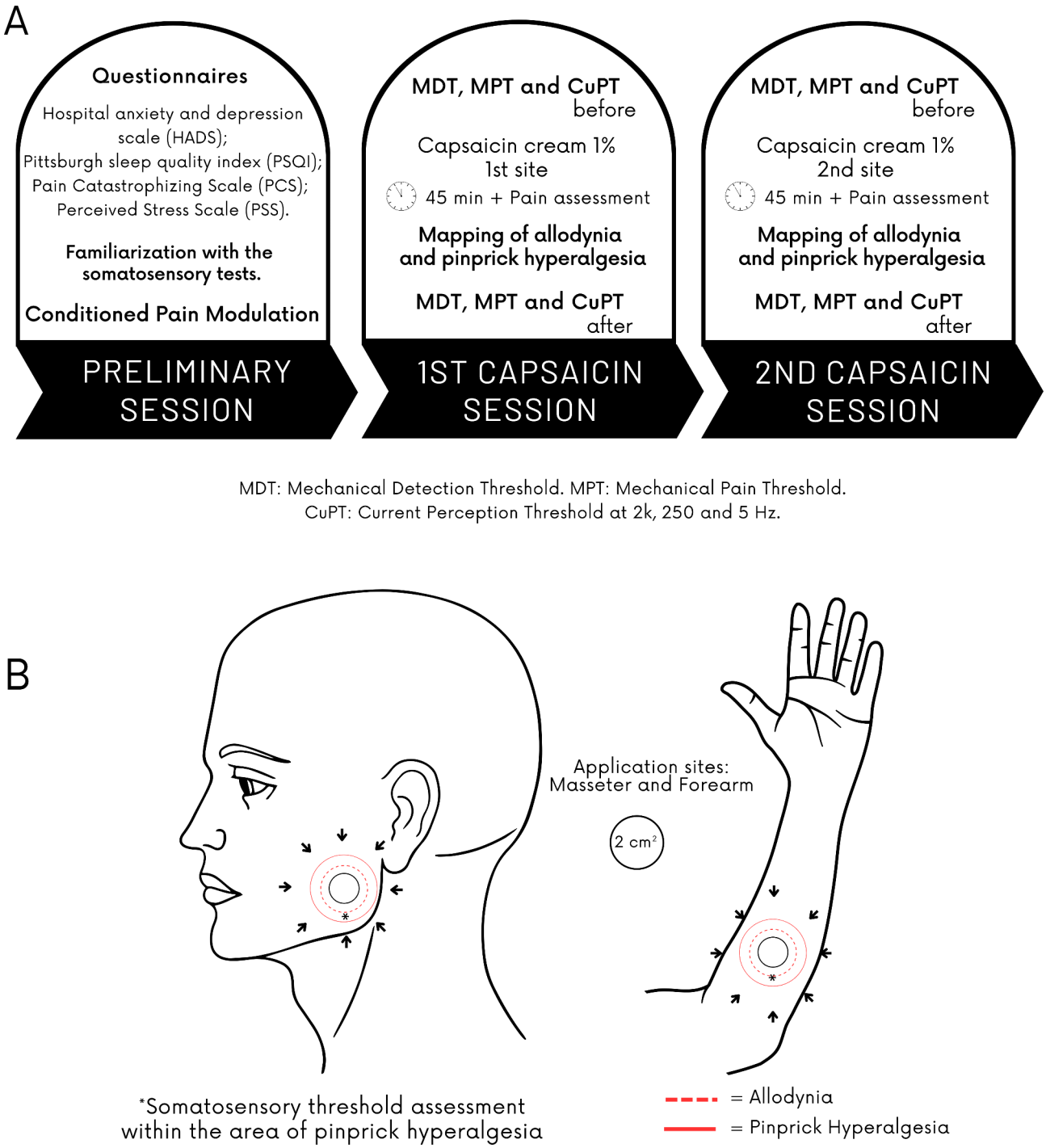

Fig. 1. Phases of the study and experimental design where 40 healthy participants (20 males and 20 females) were included and completed all the sessions. (**A**) Timeline of the study sessions and outcome variables. The washout period between sessions ranged from 24 to 72 h. (**B**) Illustration of the areas of capsaicin application and somatosensory assessment.

close as possible to the anticipated region of secondary hyperalgesia. Pain during capsaicin application was also assessed. The primary outcomes of this study were: the area of pinprick hyperalgesia and allodynia and the MPT and CuPT within the area of pinprick hyperalgesia. Secondary outcomes were pain presence and intensity during capsaicin application, MDT and the magnitude of the CPM. The following questionnaires (secondary outcomes) were also applied: Hospital anxiety and depression scale (HADS_A and HADS_D)[25], Pittsburgh sleep quality index (PSQI)[26], Pain Catastrophizing Scale (PCS)[27] and Perceived Stress Scale (PSS)[28].

The mapping of the area of dynamic mechanical allodynia started in a region distant from the capsaicin application, with a standardized soft brush (brush n.00 KZ100-00, Kaz. Sao Paulo, Brazil) moved towards the

application area at constant speed of 1 cm/s until the participant reported a painful sensation. For this mapping, eight radial lines were determined. On each ray, the point of transition from tactile sensation to pain was marked, and when interconnected, these points formed the area of allodynia[13,29]. The mapping of the area of pinprick hyperalgesia was assessed using a 180 g nylon von Frey filament (Aesthesio, DanMic Global LLC. San Jose CA, USA). Prior to the evaluation, it was confirmed that the 180 g filament induced a painful sensation. The assessment initiated in a region distant from the cream application area, and the stimuli were dynamically applied at 1 cm intervals. Similarly, eight radial lines were determined and the point at which participants reported a noticeable increase in pain sensation from the stimulus was marked. These marked points formed the area of pinprick hyperalgesia[13,29]. The demarcated areas were transferred onto tracing paper, scanned and the area was calculated using ImageJ software (US National Institute for Health). The mapping of secondary hyperalgesia spanned multiple dermatomes: C5, C6, and T1 for the forearm[30], and V1, V2, and V3 for the trigeminal region[31].

MDT and MPT tests were performed using nylon von Frey filaments (Aesthesio, DanMic Global LLC. San Jose CA, USA) and following the recommendations of the German Research Network for Neuropathic Pain (DFNS)[32]. Electrical thresholds were evaluated using the Neurometer CPT/C device (Neurotron, Baltimore, USA). We used the standard electrodes, which have a circular shape with a 1 cm^2 diameter. The CuPT evaluates the neural conduction threshold, which is the minimum electrical stimulus required to induce sensation. This test aims to independently evaluate the functional integrity of peripheral nerve fibers A-β, A-δ, and C, utilizing stimuli with frequencies of 2000 Hz, 250 Hz, and 5 Hz, respectively[33,34].

Pain intensity during capsaicin application was assessed using a 0–10 numerical rating scale (NRS), where zero indicated "no pain" and 10 indicated "the worst imaginable pain". Participants were asked about the intensity of pain in the application area at the time points 0, 5, 10, 15, 20, 25, 30, 35, 40 and 45 min. Subsequently, the mean pain intensity, area under the curve (AUC) and maximum pain intensity were calculated. Pain latency indicating the time point when the pain started was also calculated.

The magnitude of CPM was assessed using the pressure pain threshold (PPT) as the test stimulus (TS)[35]. PPT was measured utilizing a digital dynamometer (Kratos®, Cotia, Brazil) equipped with a 1 cm2 flat, circular-shaped tip[35]. Participants were instructed to press a button upon experiencing the first sensation of pain. The PPT was determined as the arithmetic mean of three trials. TS was assessed at the anterior region of the temporalis muscle (trigeminal) and the thenar muscle (spinal) of the dominant side[35]. The conditioning stimulus (CS) was the immersion of the non-dominant hand in a bucket of ice water for 2 min, maintaining a temperature between 10 and 12 °C[36]. We applied the parallel paradigm, wherein the TS was applied before (unconditioned TS) and concurrently with the CS (conditioned TS), starting after 30 s. The order of TS application sites (trigeminal and spinal) was randomized for each participant, with a 30 s interval between each region. The difference between the unconditioned TS and conditioned TS was considered the magnitude of CPM for both regions, with negative values indicating pain inhibition[18,37].

Statistics

For the sample size calculation, we estimated a large effect size (f) of 0.2 for differences in the area of pinprick hyperalgesia between the trigeminal and spinal regions. This effect size was based on previous evidence that showed a larger area of secondary hyperalgesia in the spinal region following intradermal capsaicin and sex differences in such response[15]. Thus, we considered a mixed analysis of variance (ANOVA) model with no more than two within-subject factors and one between-subject factor, a correlation between the repeated measures of at least 0.65, a power of 80%, a significance level of 5%, and an anticipated dropout rate of 10%. Therefore, the sample size calculation was at least 40 participants (20 male and 20 female participants).

Outcome variables were described using mean and standard deviation (SD) or by distribution of proportions. The normal distribution of quantitative variables was evaluated using the Kolmogorov–Smirnov test and Q–Q plots and a data transformation (log10) was applied for non-normally distributed results ($p < 0.05$). All variables were log transformed except age and CPM values.

The incidence of dynamic mechanical allodynia and pinprick hyperalgesia between the regions was compared using McNemar's test. Additionally, McNemar's test was used to compare the proportion of participants reporting pain following capsaicin between the regions, both overall and by sex. Participants who developed areas of allodynia or pinprick hyperalgesia greater than 2 cm^2 were considered responders, considering that sensory changes within the application area of capsaicin are not due to secondary hyperalgesia[14,38,39]. Finally, the spatial amplification index for the trigeminal and spinal pinprick hyperalgesia was calculated by dividing the area of pinprick hyperalgesia by the area of capsaicin application[12].

ANOVA with one within-subject factor (site, 2 levels—masseter and forearm) and one between-subject factor (sex, 2 levels—male and female) was computed to compare the area of hyperalgesia and allodynia, the intensity of capsaicin pain—AUC, mean of pain and maximum pain, and the pain latency. To assess somatosensory threshold changes, a mixed ANOVA was performed considering two within-subject factors, i.e., time (2 levels—before capsaicin and after capsaicin) and site (2 levels—masseter and forearm) and one between-subject factor sex (2 levels—male and female). Multiple comparison analyses were performed using Tukey Honest test. The significance level was set at 5% ($p = 0.050$).

T-test for independent sample was applied to compare the magnitude of CPM between those who showed a decrease in MPT and MDT (indicating hypoalgesia/esthesia) following capsaicin or those who showed an increase (indicating hyperalgesia/esthesia). The significance level was set at 5% ($p = 0.050$).

Spearman's rank correlation coefficients (rs) were calculated to measure the linear association between the magnitude of the CPM and psychosocial variables with (1) the area of secondary hyperalgesia and (2) pain intensity during capsaicin. The correlation between the area of secondary hyperalgesia and the pain intensity during capsaicin was also evaluated. A Bonferroni correction was applied due to the multiple comparisons and

the significance level was adjusted to 0.7% ($p = 0.007$), considering the number of family of associations (n = 7, CPM, capsaicin outcomes, HADS_A, HADS_D, PSQI, PCS and PSS).

Results

Areas of pinprick hyperalgesia and allodynia

All 40 individuals successfully completed the sessions, responded to the questionnaires, and none had used analgesics within the 24 h preceding the sessions. The overall mean age (SD) was 26 (4.2) years, and there was no age difference between the 20 male and 20 female participants ($p = 0.689$). The mean age (SD) was 26.1 (4.6) for male participants and 26.6 (3.9) for female participants. Table 1 describes the proportions of individuals who developed pinprick hyperalgesia and allodynia in the masseter and forearm in response to topical application of 1% capsaicin. The number of participants who developed allodynia was 10 for the masseter and 19 for the forearm. Regarding pinprick hyperalgesia, 27 participants developed it in the masseter, while 35 developed it in the forearm.

The incidence of allodynia [X^2 (1) = 7.111; $p = 0.004$] and hyperalgesia [X^2 (1) = 6.125; $p = 0.008$], evaluated using the McNemar test, was higher in the forearm than in the masseter (Table 1). Also, the areas of pinprick hyperalgesia [$F_{1,38} = 53.491$; $p = 0.000$; $\eta = 0.585$] and allodynia [$F_{1,38} = 15.087$; $p = 0.000$; $\eta = 0.284$] were larger in the forearm (Fig. 2). Moreover, no sex differences were found for either the areas of pinprick hyperalgesia [$F_{1,38} = 0.034$; $p = 0.856$; $\eta = 0.001$] or allodynia [$F_{1,38} = 0.7$; $p = 0.402$; $\eta = 0.019$].

Pain during topical capsaicin application

A total of 29 participants (72.5%, 95% CI: 58–86%) reported pain following the application of 1% capsaicin to the masseter, while 33 participants (82.5%, 95% CI: 70–94%) reported pain in the forearm. Among women, 14 (70%) reported pain in the masseter and 17 (85%) reported pain in the forearm. Among men, 15 (75%) reported pain in the masseter and 16 (80%) reported pain in the forearm. Furthermore, participants who did not report pain were not always the same as those who did not develop areas of secondary hyperalgesia. For example, nine participants reported pain but did not develop hyperalgesia, while seven participants did not report pain but did develop areas of secondary hyperalgesia in the masseter region. In the forearm, two participants reported pain without developing hyperalgesia, and four participants did not report pain but presented with secondary hyperalgesia. There was no site difference in the proportion of people who reported pain ($p > 0.050$). Additionally, only maximum pain intensity was higher at the forearm compared to the masseter during capsaicin application [*main effects* $F_{1,38} = 8.4$; $p = 0.006$; $\eta = 0.182$, Table 2]. However, when analyzed by sex, such difference was observed only for female participants [*interaction between site and sex* $F_{1,38} = 6.9$; $p = 0.012$; $\eta = 0.155$, Table 2].

Pain latency following capsaicin application was greater for the forearm when compared with the masseter (*main effects* $F_{1,38} = 6.975$; $p = 0.012$; $\eta = 0.155$, Table 2) for both male and females, which means no significant interaction between site and sex. Capsaicin-induced pain ratings assessed every 5 min during the 45-min exposure period are presented in Fig. 3. A moderate and significant linear correlation was observed between the area of pinprick hyperalgesia following capsaicin application and pain intensity during capsaicin application, but this difference was observed only at the forearm [$rs = 0.426$; $p = 0.006$, Fig. 4A and B].

Somatosensory threshold assessment

The mean (SD) and 95% CI of the mean for the MDT, MPT and the three frequencies of CuPT (2 k, 250 and 5 Hz) are described in Table 3. MDT values after the application of capsaicin were significantly greater than MDT values before capsaicin, and this difference was observed only at the forearm (Tukey, $p = 0.043$, Table 3). MPT values after the application of capsaicin were significantly greater than MPT values before capsaicin, but this difference was observed only at the masseter (Tukey $p = 0.019$, Table 3). Moreover, MPT values after the application of capsaicin were significantly higher at the masseter compared to the forearm values after capsaicin (Tukey, $p < 0.001$, Table 3).

CuPT 2 kHz assessment indicated that values were higher at the masseter when compared with the forearm (Tukey, $p < 0.001$, Table 3). CPT 250 Hz values were also higher at the masseter when compared with the forearm values at all assessment times (Tukey, $p < 0.001$, Table 3). In addition, CuPT 250 Hz values after the application of capsaicin were significantly greater than CuPT 250 Hz values before capsaicin, but this difference was observed only at the masseter (Tukey $p < 0.001$, Table 3), CuPT 5 Hz values after the application of capsaicin were significantly greater than CuPT 5 Hz values before capsaicin, but this difference was observed only at the

	Allodynia				**Pinprick Hyperalgesia**			
	Masseter		***Forearm***		***Masseter***		***Forearm***	
	N (%)	**95% CI**	**N (%)**	**95% CI**	**N (%)**	**95% CI**	**N (%)**	**95% CI**
Female *(N = 20)*	7 (35%)	(15–59%)	11 (55%)	(31–76%)	13 (65%)	(40–84%)	18 (90%)	(68–98%)
Male *(N = 20)*	3 (15%)	(3.2–37%)	8 (40%)	(19–63%)	14 (66.7%)	(45–88%)	17 (81%)	(62–96%)
Total *(N = 40)*	10 [a] (25%)	(12–41%)	19 (47.5%)	(31–63%)	27 [b] (67.5%)	(50–81%)	35 (87.5%)	(73–95%)

Table 1. Percentages and their 95% confidence interval (CI) of participants who developed allodynia and pinprick hyperalgesia following the topical application of 1% capsaicin on the masseter and forearm. [a]Significant differences between sites, McNemar [X^2 (1) = 7.111; $p = 0.004$]. [b] Significant differences between sites, McNemar [X^2 (1) = 6.125; $p = 0.008$]. N = number of participants.

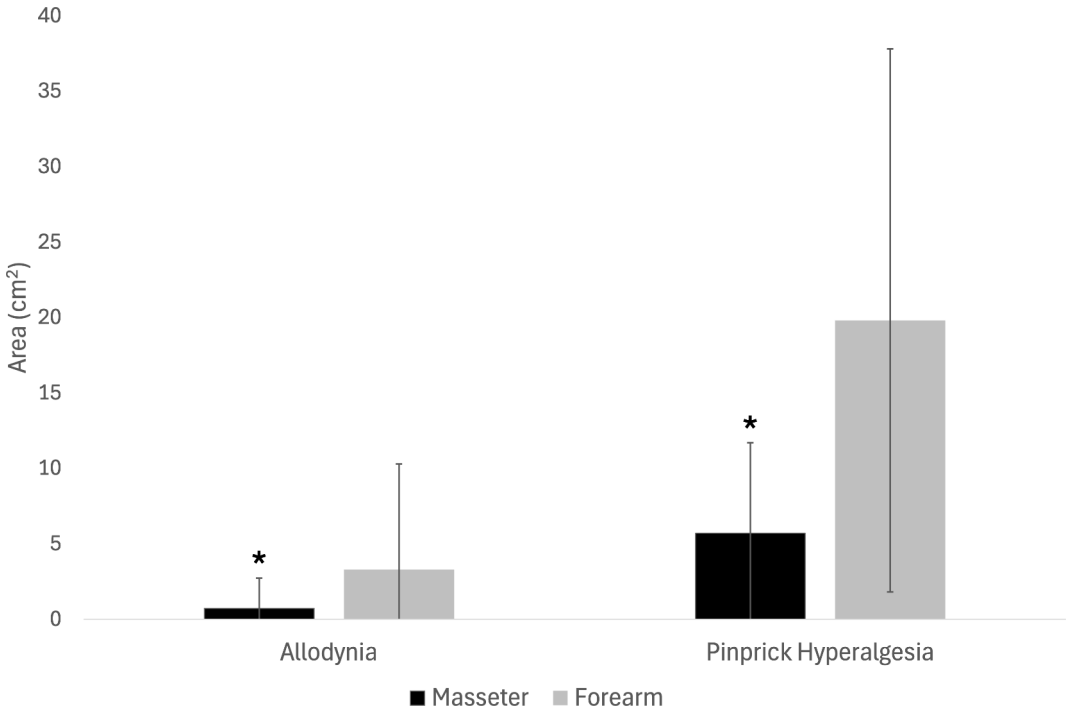

Fig. 2. Mean of allodynia and pinprick hyperalgesia areas developed in response to topical application of 1% capsaicin to the masseter and forearm (n = 40). Error bars indicate the standard deviation of the mean. * indicates significant differences between the masseter and forearm ($p < 0.050$).

		Masseter			Forearm		
		Female	Male	Total	Female	Male	Total
Average Pain (0–10 NRS)	Mean	1.8	1.1	1.1	1.3	1.3	1.3
	SD	1.3	1.0	1.2	1.1	1.2	1.2
	95% CI	0.5–1.8	0.7–1.6	0.8–1.5	0.8–1.9	0.7–1.8	0.9–1.7
AUC (0–10 NRS SUM)*	Mean	11.8	11.3	11.5	13.5	12.8	13.1
	SD	13.4	10.2	11.8	11.5	12.2	11.7
	95% CI	5.5–18.1	6.5–16.1	7.8–15.3	8.1–18.8	7.1–18.5	9.4-–16.9
Maximum Pain (0–10 NRS)	Mean	2.7 [a]	2.2	2.5 [b]	3.7	3.0	3.3
	SD	2.5	1.8	2.2	2.4	2.2	2.3
	95% CI	1.5–3.9	1.4–3.1	1.8–3.2	2.5–4.8	1.9–4.0	2.6–4.1
Pain Latency (min)	Mean	9.2	9.7	9.5 [c]	19.7	21	20.3
	SD	4.3	6.9	5.7	8.7	8.9	8.7
	95% CI	6.7–11.7	5.7–13.8	7.3–**11**	16.1–25.7	1.9–4.0	17.2–23.4

Table 2. Mean, standard deviation (SD) and 95% confidence interval (CI) of the mean for average pain intensity, area under the curve (AUC), maximum pain intensity and pain latency following the topical application of 1% capsaicin on the masseter and forearm (n = 40). [a]Significant difference between sites, ANOVA [F = 6.9; $p = 0.012$]. [b] Significant differences between sites, ANOVA [F = 8.4; $p = 0.006$]. [c] Significant differences between sites, ANOVA [F = 43.5; $p < 0.001$]. * The area under the curve was calculated as the sum of pain intensities at each assessment time throughout the entire duration of capsaicin application. NRS = numeric rating scale.

masseter (Tukey $p < 0.001$, Table 3). Finally, CuPT 5 Hz values were highest after capsaicin at the masseter in relation to values before capsaicin (Tukey, $p < 0.001$, Table 3). Finally, the spatial amplification index for pinprick hyperalgesia was 9.9 in the forearm and 2.8 in the masseter.

CPM and psychosocial assessment

The mean (SD) of unconditioned and conditioned TS at the temporalis were 3.12 (1.6) and 3.25 (1.5) kgf/cm^2, respectively. Consequently, the mean (SD) of the magnitude of CPM at the trigeminal region was −0.13 (0.5),

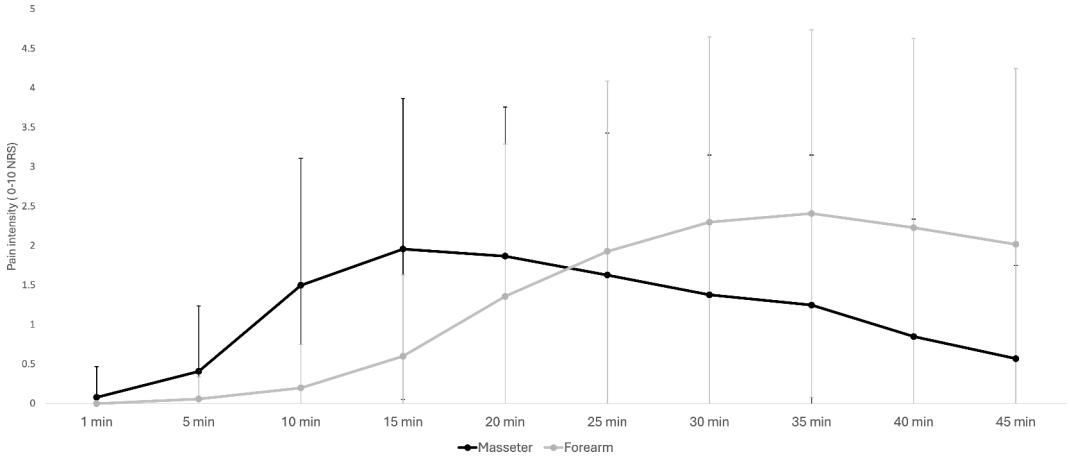

Fig. 3. Mean pain intensities recorded every 5 min over the 45-min application of 1% topical capsaicin on the masseter and forearm (n = 40). Error bars indicate the standard deviation of the mean. NRS = numeric rating scale.

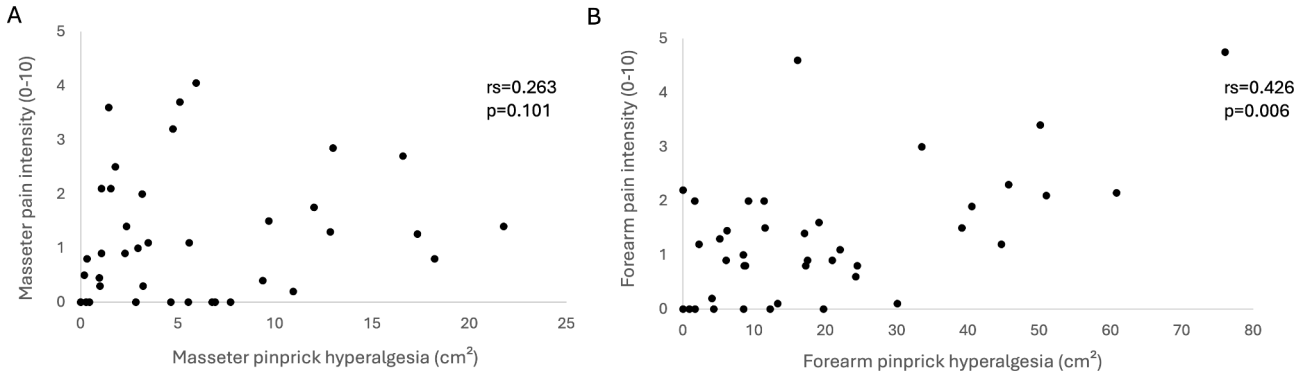

Fig. 4. Correlation between the area of pinprick hyperalgesia and average pain intensity following application of 1% topical capsaicin. (**A**) Corresponds to masseter evaluation (trigeminal region); (**B**) Refers to assessment on forearm (spinal region).

with a 95% CI of − 1.15 to 1.50 kgf/cm^2. Similarly, the mean (SD) of the unconditioned and conditioned TS at the thenar were 5.28 (1.9) and 5.74 (1.6) kgf/cm^2, resulting in a CPM mean (SD) magnitude at the spinal region of − 0.46 (1.2), and a 95% CI of − 2.76 to 2.64 kgf/cm^2. The overlapping 95% CIs suggest no significant differences between the two regions. Moreover, the CPM magnitude at the trigeminal region between participants who showed an increase in trigeminal MPT [n = 26, mean (SD) of − 0.19 (0.44) kgf/cm^2] was no different from those who showed a decrease in trigeminal MPT [n = 12, mean (SD) of − 0.12 (0.45)], ($p = 0.637$). The CPM magnitude at the trigeminal region did not show differences between participants who showed an increase in trigeminal MDT [n = 26, mean (SD) of -0.15 (0.47)] when compared with those who showed a decrease [n = 12, mean (SD) of − 0.05 (0.58)] ($p = 0.565$). Likewise, the CPM magnitude at the spinal region between participants who showed an increase in spinal MPT [n = 20, mean (SD) of − 0.53 (1.08)] was no different from those who showed a decrease in spinal MPT [n = 20, mean (SD) of − 0.38 (1.32)] ($p = 0.992$). The CPM magnitude at the spinal region did not show differences between participants who showed an increase in spinal MDT [n = 28, mean (SD) of − 0.52 (1.21)] when compared with those who showed a decrease [n = 12, mean (SD) of − 0.31 (1.18)] ($p = 0.497$).

There was no significant linear correlation between the magnitude of CPM at the trigeminal area and either the area of pinprick hyperalgesia following capsaicin application or pain intensity during capsaicin application at the masseter (Fig. 5A and D). Likewise, there was no significant linear correlation between the magnitude of CPM at the spinal area and the area of pinprick hyperalgesia or pain intensity following capsaicin application at the masseter (Fig. 5C and D).

Table 4 presents the mean (SD), and 95% CI of the mean for the psychosocial questionnaires. No significant correlations were found between psychosocial variables and the area of pinprick hyperalgesia or pain intensity (see Supplementary Figures S1–S5).

		Masseter		Forearm	
		Bef	*Aft*	*Bef*	*Aft*
MDT (g/mm^2)	Mean	0.23	0.36	0.27	0.48 *
	SD	0.83	0.68	0.42	0.84
	95% CI	−0.03–0.50	0.13–0.57	0.13–0.40	0.21–0.75
MPT (g/mm^2)	Mean	48.85	61.59 *#	68.94	50.43
	SD	84	86	118	88
	95% CI	22.00–75.69	34.00–89.03	31.06–106.82	22.38–78.46
CuPT 2 k (μA)	Mean	145.17 #	151.69 #	82.12	88.4
	SD	53	61	33	33
	95% CI	128.15–162.19	132.21–171.21	71.53–92.71	77.87–98.92
CuPT 250 (μA)	Mean	48.39 #	58.62 #	32.57	35.1
	SD	25	27	12	12
	95% CI	40.44–56.24	50.12–67.12	28.86–36.28	31.26–39.00
CuPT 5 (μA)	Mean	36.9	52.8 *#	25.15	30.07
	SD	24	25	13	12
	95% CI	29.35–44.44	44.66–60.93	21.13–29.00	26.10–34.04

Table 3. Mean, standard deviation (SD) and 95% confidence interval of the mean (95% CI) of the raw data for the mechanical detection threshold (MDT), mechanical pain threshold (MPT) and current perception threshold (CuPT) at three frequencies (2 kHz, 250 Hz and 5 Hz). Assessment times were before (Bef) and after (Aft) the topical application of 1% capsaicin, administered at two sites (masseter and forearm) during two separate sessions (n = 40). * = indicates significant mean differences between the assessment times (before and after capsaicin) for the same site, either masseter or forearm ($p < 0.050$). # = indicates significant mean differences between sites (masseter and forearm) for the same assessment time, either before or after capsaicin ($p < 0.050$). The acronym A corresponds to ampere, a unit of current that is equivalent to Coulomb per second. Ampere is a very large unit, so when stimulation is performed on humans, smaller units are used, such as μA.

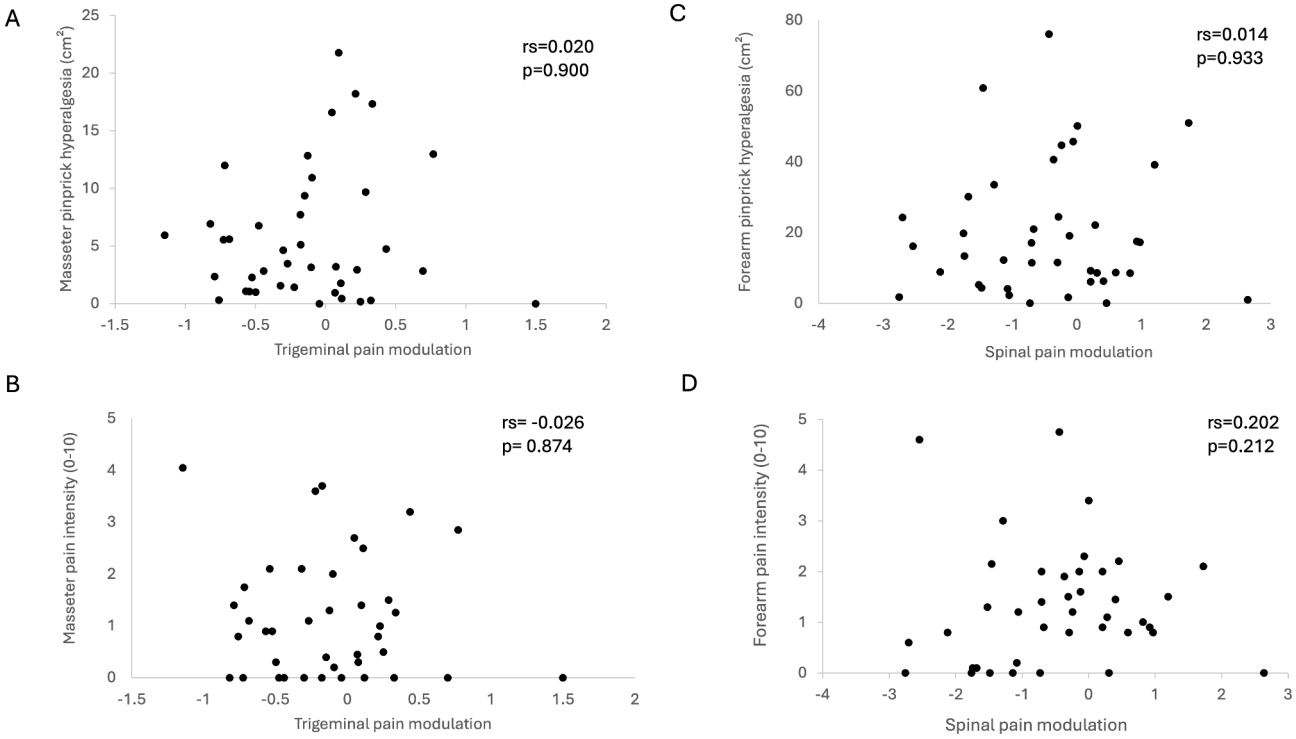

Fig. 5. Correlation between conditioned pain modulation magnitude and area of pinprick hyperalgesia, and average pain intensity following 1% topical capsaicin on the masseter and forearm. (**A**) and (**B**) refer to application of the test stimulus at the anterior region of the temporalis muscle (trigeminal pain modulation). (**C**) and (**D**) refer to application of the test stimulus at the thenar region (spinal pain modulation).

	HADS_A	HADS_D	PSQI	PCS	PSS
Mean	7.65	3.55	6.55	10.5	31.25
SD	2.89	2.37	3.64	7.09	3.17
95% CI	5.82–9.47	2.43–4.66	4.84–8.25	7.17–13.82	29.76–32.73
Mean	7.1	5.65	6.15	9.1	29
SD	3.61	3.80	2.39	8.92	3.97
95% CI	5.4–8.79	3.87–7.42	5.03–7.26	4.92–13.27	27–30.85
Mean	7.37	4.6	6.35	9.8	30.12
SD	3.71	3.3	3.05	7.98	3.72
95% CI	6.18–8.56	3.5–5.65	5.37–7.32	7.24–12.35	28.93–31.31

Table 4. Mean, standard deviation (SD) and 95% confidence interval of the mean (95% CI) of the scores of the following psychosocial questionnaires: hospital anxiety and depression scale and (anxiety HADS_A, depression HADS_D), Pittsburgh sleep quality index (PSQI), pain catastrophizing scale (PCS) and perceived stress scale (PSS) (n = 40).

Discussion

This study investigated somatosensory differences between the trigeminal and spinal innervation in response to topical capsaicin. The results indicate a novel distinction between the two pathways concerning capsaicin-evoked secondary hyperalgesia. It was demonstrated that the extent of capsaicin-evoked secondary hyperalgesia in the trigeminal region was significantly smaller compared to the spinal region, which usually present larger areas of secondary hyperalgesia[12]. Moreover, we observed a slight decreased sensitivity following the capsaicin application in the trigeminal region. Therefore, these main findings suggest that manifestations compatible with central sensitization due to capsaicin exposure seem to be less pronounced in trigeminal region when compared with spinal region.

The findings that support a less robust expression of central sensitization related to secondary hyperalgesia in trigeminal region are the differences in the incidence and the extent of allodynia and pinprick hyperalgesia between the two regions. A lower proportion of participants developed allodynia following capsaicin application in trigeminal (25%) when compared with the spinal region (47.5%). Likewise, the proportion of pinprick hyperalgesia also differed between the two regions, with a lower proportion in the trigeminal (67.5%) when compared with the spinal region (87.5%). Furthermore, the areas of allodynia and pinprick hyperalgesia were 0.7 and 5.7 cm^2 for the trigeminal region, respectively. In contrast, for the spinal region, these areas were 3.29 and 19.8 cm^2, representing 4.7 and 3.4 times larger, respectively. Participants who did not report pain were not necessarily the same as those who did not develop areas of secondary hyperalgesia. This suggests that, for some individuals, the nociceptive input from topical capsaicin application may be sufficient to induce signs of secondary hyperalgesia, even if it is not strong enough to be perceived as painful.

The size of the capsaicin application area and the subsequent pinprick hyperalgesia exhibit significant variability across studies according to a recent systematic review[12]. To facilitate comparative analysis between studies, a spatial amplification index is calculated, representing the ratio between the area of pinprick hyperalgesia and the area of capsaicin application. The mean index is 17.6 for topical capsaicin in the spinal region with a 95% CI ranging from 7 to 28[12]. In the present study, the spatial amplification index was 9.9 for the spinal region, in agreement with previous studies[12]. In contrast, the trigeminal region yielded an amplification index of only 2.8, indicating relatively minor sensitization effects of topical capsaicin.

The smaller area of secondary hyperalgesia in the trigeminal region compared to the spinal region has been previously reported following intradermal capsaicin application[15]. This finding may be partly explained by the smaller receptive field size of central neurons in the trigeminal spinal nucleus compared to those in the dorsal horn[15]. However, our results also demonstrated a loss of function in specific somatosensory modalities in the trigeminal region following capsaicin. For MPT, values increased solely after capsaicin at the masseter, indicating pinprick hypoalgesia. Similarly, the values for CPT 250 Hz and CPT 5 Hz, which assess the neuronal conduction of A-δ fibers and C fibers, respectively, exhibited higher thresholds after capsaicin application at masseter. The direction of somatosensory threshold changes in MPT following capsaicin application in the area of secondary hyperalgesia in the trigeminal region was unexpected and may appear contradictory. However, these findings could be attributed to methodological differences between the assessments. Secondary hyperalgesia mapping involved a dynamic mechanical assessment across different locations, while the MPT test provided a static assessment at a single site. Moreover, previous studies using lower concentrations of capsaicin applied intraorally for 15 min also showed either no change or reduced pain sensitivity to mechanical stimuli outside the application area[40,41]. Overall, the low incidence and limited extent of allodynia and pinprick hyperalgesia, along with a slight reduction rather than an increase in somatosensory function within the area of secondary hyperalgesia, may reflect physiological specificities of the trigeminal region in response to topical capsaicin.

It has been demonstrated that mechanical pain sensitivity is increased within the area of secondary hyperalgesia following topical capsaicin application to the forearm[24]. However, there is also evidence indicating minor and non-significant changes in sensitivity within the same area[42]. Likewise, we did not observe a significant reduction in MPT values at the forearm. This finding can be attributed to the comparatively less robust effects of topical capsaicin when compared to intradermal capsaicin[12]. For MDT, values were higher after capsaicin only at the forearm, indicating hypoesthesia, an observation consistent with prior findings in the literature[43].

Although further studies are needed to reproduce these findings and to explore in detail the mechanisms underlying these differences, some possibilities are candidates to further hypothesis-driven studies. For example, there is evidence that skin thickness could alter capsaicin permeability[44], thus permeability differences between the regions are possible. TRPV1's responsiveness difference is also a likely explanation. This receptor, in contact with capsaicin, can be sensitized and/or desensitized depending on the intensity and duration of stimulation, the location of the receptor and the period between stimuli[45]. Considering that the application area and the amount of capsaicin were identical between sites, it is possible that the effect of topical capsaicin 1% for 45 min generates desensitization in face and sensitization in forearm. Furthermore, there seems to be different isoforms of TRPV1, one found throughout the body and another expressed only in neurons that innervate the face[7]. The overall pain experience during capsaicin application did not differ between regions, as no significant differences were observed in mean pain intensity or AUC of pain intensity. However, differences were noted for maximum pain intensity in females and pain latency, where the forearm exhibited higher maximum pain intensity values in females and a longer pain latency compared to the masseter, suggesting potential peripheral differences in response to capsaicin. The overall lower pain thresholds in the facial region, relative to the upper limb[32], may explain the longer pain latency, while the lower proportion of unmyelinated fibers in the trigeminal nerve compared to the spinal nerve[4] may account for the relative higher maximum pain intensity in the forearm. This higher pain intensity was observed only in females, underscoring potential sex differences in pain perception in response to topical capsaicin[46]. Nonetheless, we believe that peripheral factors alone may not fully account for the observed differences and that some degree of overlap with central mechanisms is likely. The key distinctions were in the incidence, extent, and sensory changes within the area of secondary hyperalgesia, which are compatible with central sensitization[21,47].

Allodynia and pinprick hyperalgesia following capsaicin occur through facilitation of myelinated fibers, A-β and A-δ, respectively[14]. However, it is important to emphasize that pain facilitation and inhibition coexist, and the outcome depends on the proportion between them[48]. Consequently, it is plausible that trigeminal innervation exhibits an increased threshold to pain facilitation or presents more efficient pain inhibition compared with spinal innervation in response to capsaicin. Despite descending GABAergic modulation appearing less robust in trigeminal pathways compared to spinal pathways[49], the blockade of rostral ventromedial medulla completely inhibited mechanical allodynia in the forehead, while it only partially and temporarily blocked allodynia in the hind paw of rats[50]. Furthermore, there is clinical evidence suggesting that CPM seems to be less potent when assessed in the face using thermal stimuli[22]. However, the latter has not been consistently reproduced, and they could be explained by behavioral mechanisms not necessarily related to descending pain inhibition, as assessed by the CPM[22]. Overall, these findings suggest that mechanisms for pain modulation may not operate uniformly between trigeminal and spinal pathways. Further studies are needed to replicate our findings and conclusively establish differences in pain facilitation and inhibition between these regions.

The CPM magnitude was not associated with the extent of pinprick hyperalgesia or with pain intensity during capsaicin application. Similarly, CPM magnitude did not differ between participants who showed increased or decreased MPT or MDT following capsaicin application in either the trigeminal or spinal regions. We followed the original CPM practice recommendations, where the painfulness of the conditioned TS is subtracted from that of the unconditioned TS[18]. However, this approach does not account for potential measurement errors and natural fluctuations, which may partly explain our lack of significant associations and differences. In fact, recent investigations have recommended protocols that allow for a calculation of a "net effect" to increase the reliability and clinical relevance of CPM assessment[51,52]. On the other hand, we found a moderate correlation between the area of secondary hyperalgesia and pain intensity in the forearm, further suggesting a more robust and consistent response to capsaicin.

We did not find any significant linear associations between psychosocial questionnaire scores and the degree of secondary hyperalgesia or pain intensity during capsaicin exposure, based on the adjusted p-value. A prior study using regression analysis similarly reported no linear association between pain catastrophizing and either the area of secondary hyperalgesia or pain intensity[53]. However, it did observe moderation effects when participants were categorized into different pain catastrophizing clusters[53]. These findings underscore the complex relationship between psychosocial factors and pain outcomes, which can vary depending on data curation methods. Future studies are warranted to investigate this issue more systematically.

Our findings have both clinical and research implications. For instance, it has been suggested that trigeminal innervation has a lower tendency to develop post-traumatic neuropathic pain[6,11]. Thus, our results may support the proposition that traumatic trigeminal neuropathies are less common. This interpretation is based on the validation of capsaicin application as an experimental pain model for investigating manifestations of central sensitization related to secondary hyperalgesia in the development of neuropathic pain[21,54–56]. Furthermore, our findings can improve the understanding of chronic orofacial pain and contribute to the development of new drugs and targeted therapies for orofacial pain. Finally, given the challenges of exploring the clinical manifestation of central pain mechanisms—which often require advanced techniques such as neuroimaging and electrophysiology—the capsaicin model offers a feasible and relatively cost-effective tool for investigating these mechanisms in the orofacial region.

Although the area of hyperalgesia in the forearm developed as expected[12], one of the limitations of our study was the application of only topical capsaicin, a less robust model for the development of pain, hyperalgesia and allodynia when compared to injectable. Evidence suggests that combining heat with topical capsaicin may induce longer lasting and more stable sensitization effects, highlighting its potential for future investigations[57,58]. However, due to technical constraints, we were unable to apply the heat/capsaicin model in this study. Other limitations were the absence of a control intervention such as a nocebo cream, additional somatosensory assessment, such as thermal thresholds and the lack of delimitation of the flare area. Delimiting the flare area would be important to exactly demarcate the area of the secondary hyperalgesia[8]. Nonetheless, the extension

of the secondary hyperalgesia area for the spinal region was similar to what has been reported[12]. Furthermore, the lack of flare demarcation possibly contributed to an overestimation of the area of secondary hyperalgesia[8]. That is, it is possible that future studies where this methodology is reproduced can find similar or more robust differences. The absence of pre-registration is a limitation of this study, and future pre-registered investigations are needed to confirm the observed differences.

In conclusion, we observed notable differences between the trigeminal and spinal regions in response to topical capsaicin, with the trigeminal region appearing less susceptible to sensitization effects. These findings suggest potential differences compatible with central sensitization related to secondary hyperalgesia between the trigeminal and spinal innervation.

Data availability

Original datasets generated over the course of this research are available from the corresponding author on reasonable request.

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Competing interests

The authors declare no competing interests.

Additional information

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