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ORIGINAL ARTICLE

Effect of heterotopic noxious conditioning stimulation on electrical and pressure pain thresholds in two different anatomical regions

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Abstract

Objectives. The aims of the study were to investigate the influence of heterotopic noxious conditioning stimulation (HNCS) on pain thresholds in the orofacial and spinal regions and to find out whether there are gender differences in this respect. ***Material and Methods.*** Thirty healthy subjects (15 of each sex) with a mean (SD) age of 25.1 (4.4) years participated. Pain thresholds to electrical (EPT) and pressure stimuli (PPT) were recorded in the masseter muscle and 1st upper incisor (tooth), as well as in the fingertip, before, during, and 5 and 15 min after a cold pressor task to the contralateral hand immersed in ice-cold water for a maximum of 5 min. ***Results.*** With the exception of the EPT in the orofacial region, all pain thresholds increased during the HNCS and then returned to baseline during the 15 min follow-up. The significant changes in EPT were greater in the finger than in the tooth, while the changes in PPT were greater in the masseter muscle than in the finger. Electrical stimuli in the finger induced greater significant changes of pain thresholds than pressure. In the orofacial region, pressure induced greater significant changes in pain thresholds during HNCS than electrical stimuli did. The HNCS induced pain of high intensity and unpleasantness, i.e. varying between 5 and 10 on the numeric rating scale (NRS). There were no gender differences in the response to the HNCS. ***Conclusion.*** We conclude that, in general, HNCS induced by cold pressor stimulation increases pain thresholds, but the magnitude of the effect differs between the orofacial region and the finger and is influenced by the tissue and type of test stimuli.

Key Words: *Electrical stimulation, gender differences, heterotopic noxious conditioning stimulation (HNCS), mechanoreceptors, pain thresholds*

Introduction

Pain transmission is controlled by both spinal and supraspinal mechanisms. Activation of large diameter afferent fibers inhibits pain transmission in the dorsal spinal cord via inhibitory interneurons. Supraspinally, pain is modified by the endogenous pain inhibitory system acting via descending pathways from the brainstem to the dorsal spinal cord [1]. The concept of diffuse noxious inhibitory control (DNIC) is often used in experiments on animals [2–4].

Pain modulation is believed to be mediated by mechanisms acting on wide dynamic range (WDR) neurons in the spinal dorsal horn and in the trigeminal nucleus caudalis. Experiments on animals have shown that these neurons receive important A-fiber and C-fiber input and can be activated by thermal, chemical, electrical, and mechanical noxious activation of muscle and cutaneous afferent [5].

One way to activate the human endogenous pain inhibitory system experimentally is to apply a painful stimulus (conditioning stimulus) and then assess the pain induced by another noxious stimulus (test stimulus). If the conditioning stimulus is applied at a remote site, the term heterotopic noxious conditioning stimulation (HNCS) is often used. HNCS has been used extensively to experimentally study pain modulation in the endogenous pain inhibitory pathways in humans [6,7].

Reports conflict on whether there are gender differences with respect to pain modulation. Some studies report that HNCS is less efficient in women than in men [8–11], others do not show any gender differences at all [12–14]. There is evidence from

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human experimental studies that stimulus type and duration affect pain perception and that stimuli inducing deep tonic pain reflect the largest gender differences [15–17]. The various conditioning used (e.g. hypertonic saline, cold pressor, and ischemic) and test stimuli (e.g. superficial heat and electrical as well as deep pressure) might explain the contradictions in the results between these studies. For example, electrical stimulation is receptor unspecific and directly activates both non-nociceptive and nociceptive afferents (Aβ, Aδ, and C-fibers). Pressure stimulation, on the other hand, activates polymodal mechanosensitive receptors located on Aδ and C-fibres [18]. Nociceptive information from deep tonic stimulation is subjected to stronger descending inhibition than more superficial stimulation [19], which might indicate that pressure stimulation is suppressed more efficiently than electrical stimulation.

It has been shown that the orofacial region is more densely innervated than other regions of the body [20]; for example, the pressure pain threshold (PPT) increased in healthy trapezius and tibialis muscles but not in masseter and temporalis muscles after systemic administration of the 5-HT_3 antagonist granisetron [21]. However, as in chronic myalgia, increased levels of neuropeptide Y in the trapezius muscle during ischemic conditioning have been reported, but not in the masseter muscle [22]. It is unknown whether this depends on the orofacial pain signals ascending via the medullary tract instead of the hypothalamic tract, as it does for pain signals in the rest of the body.

Since stimulus modality and anatomical location may influence pain modulation – the results conflict as to whether there are gender differences in pain modulation – we aimed at investigating the contribution of these factors on HNCS effects. Our hypotheses were: 1) Pain modulation is less efficient in the orofacial region than in the spinal region; 2) endogenous pain modulation is more susceptible to pain evoked by pressure stimulation than by pain evoked by electrical stimulation; and 3) pain modulation is less efficient in females than it is in males. For this purpose, the PPT and the electrical pain threshold (EPT) were recorded and compared in the orofacial region and the finger (spinal region) before, during, and after a cold pressor test (CPT) to the contralateral hand in healthy subjects of both genders.

Material and methods

Subjects

The study comprised 15 healthy women with a mean (SD) age of 24.5 (4.3) years and 15 age-matched healthy men with a mean (SD) age of 25.8 (4.7) years. They had no ongoing pain and had not taken any analgesics, tranquilizers, antidepressants, or other medication that could influence pain perception on the day of the study.

The study followed the principles of the Declaration of Helsinki. Subjects received both written and verbal information about the study and gave their verbal consent prior to inclusion. The methods and selection of patients were approved by the local ethics committee in Stockholm, Sweden (2005/641-31/14).

Recordings of electrical pain thresholds

EPTs in the orofacial region were recorded over one of the central maxillary incisors. An electrical pulp tester was used, commonly used for measuring sensibility in teeth (Vitality scanner; Analytic Technology, Redmond, WA, USA; range 0–80 μA, pulse rate intervals 0–9). It comprised a probe with a surface area of 2 mm through which electrical impulses fire towards the tooth when there is a closed circuit between the device, the operator, and the subject. The probe was connected to a power supply and a pulse rate interval of 5 was chosen. A conductive paste was used to get optimal connection between the probe surface and the tooth surface. The subjects were instructed to say "stop" as soon as they first experienced the first sensation of pain (pain threshold). As soon as the test stopped, the numbers froze on the display and the examiner recorded the value. To increase reliability, the recordings were measured three times before the CPT, twice during the CPT, and again three times after the CPT.

The EPT over the fingertip were recorded with PainMatcher (PainMatcher AB, Lund, Sweden; score 0–99), which is a microprocessor that distributes constant current to the electrodes; 15 mA, in monophasic rectangular pulses at random velocity with a frequency of 10 Hz. To create an electrically closed circuit, the electrodes of the instrument are pressed with the thumb and second finger of the hand [23]. By increasing the pulse width from 4 to a possible maximum of 396 μs in increments of 4 μs (i.e. 99 steps in all), the intensity of the stimulus increases. The subjects were asked to drop the device when the sensory or pain thresholds were reached. This was repeated twice and the scores for the three recordings were displayed on the screen.

Assessments of pressure pain thresholds

The PPTs, which have been reported to reflect mainly pressure pain sensitivity of deeper tissues [24], were recorded with an algometer in the masseter muscle and on the finger. The algometer (Somedic Sales AB, Hörby, Sweden) consisted of a pistol grip and a rod with a pressure-sensitive strain gauge at the tip (diameter 5 mm) connected to a power supply, an amplifier, and a display. It was

hand-held, and pressure at a rate of 30 kPa/s was increased manually [25]. The subjects were instructed to push a button to interrupt the stimulus as soon as the pressure sensation became painful. In the orofacial region the recordings were made over the most prominent part of the superficial masseter muscle with the muscle at rest; the algometer was held perpendicular to the surface of the skin. The PPT was recorded over the tip of the middle finger with the aid of a pinch handle attached to the algometer. The pinch handle allows the fingertip to be pressed against the handle by the probe during recording. The PPT was recorded three times before and after the CPT and twice during the CPT.

Heterotopic noxious conditioning stimulation

HNCS was induced by a cold pressor task where the subjects immersed their entire hand in ice-cold water (2–4°C), constantly circulating the hand in the water to avoid warming up the water in close vicinity to the hand by the body temperature. The subjects were instructed to immerse their hand in the water for a maximum of 5 min, but could remove it at any time if the pain became intolerable.

Assessments of pain

During HNCS, the subjects were asked to score the intensity and unpleasantness of the painful sensation on an analog numeric rating scale (NRS) scored 0–10 (0 = no pain and 10 = unbearable pain). The tolerance time, i.e. the time the subject could endure keeping the hand in the cold water, was also recorded.

General procedures

The subjects were seated in a conventional dental chair in a relaxed position. Before the experiment started, they were subjected to an initial training session on the side opposite to the test side. The experiment was performed in a randomized manner concerning which side was tested and the order of the regions (orofacial and finger). All subjects received a patient number (1–15 consecutively in each group). For those with even subject numbers, the right side was used for the test stimuli and the recordings started in the orofacial region (with electrical stimulation followed by PPT), while for subjects with uneven subject numbers the left side was tested and the recordings started in the finger (with PPT followed by electrical stimulation). After recordings of baseline pain thresholds, the conditioning stimulus was applied to the contralateral side. Thirty seconds and 5 and 15 min after the start of the HNCS, the pain thresholds were again recorded in the same order as during baseline recordings.

Data analysis

The average of the recordings of pain thresholds at each time-point (before, during, and 5 and 15 min after HNCS) was calculated. In order to be able to compare between changes in EPT and PPT, the data were then normalized (%) to baseline by dividing the averaged values during and after HNCS by their respective averaged values at baseline, and then multiplying by 100.

Statistics

Data are presented as mean (SD), unless otherwise stated, and were analyzed with Sigma Stat version 3.1 (Systat Software Inc). They were tested with the Kolmogorov-Smirnov test and found to be normally distributed.

The significance of the differences between genders in baseline thresholds was tested with Student's unpaired *t*-test. Two-way repeated measures ANOVA was used to test the significance of the changes in pain threshold during the experiment. Separate ANOVAs were performed to test for the influence of gender, region, and stimulus on pain thresholds, i.e. as the independent factors (two levels: males and females; orofacial and finger; electrical and mechanical, respectively). Time (four levels: baseline, during, and 5 min and 15 min after HNCS) was the repeated factor. The Holm–Sidak method for multiple comparison procedure versus a control group (baseline) was used as post-hoc test. Additional two-way repeated measures ANOVAs were used to test whether the changes in pain thresholds during and after the HNCS were influenced by the side tested (right or left) or by the order of test sites, i.e. whether the finger or orofacial region was tested first. Differences in NRS pain variables between genders were tested with the Mann-Whitney U-test, while differences in pain endurance were tested with the unpaired *t*-test. The Spearman correlation test (NRS pain variables) and Pearson's product-moment correlation test (pain endurance) were used for correlation analyses between pain variables and changes in pain thresholds. To control for multiple correlation analyses, the *p*-values were corrected according to Bonferroni. A *p*-value of <0.05 was considered significant.

Results

Baseline pain thresholds

The baseline pain thresholds are given in Table I. The EPT in the tooth and PPT in the finger were significantly lower in females than in males (Student's unpaired *t*-test; $p=0.014$ and $p=0.003$, respectively). There was also a tendency to a lower EPT in the finger in females ($p=0.055$). There were

Table I. Mean (SD) electrical and pressure pain thresolds of the finger and orofacial region in 30 healthy subjects during and 5 min as well as 15 min after heteortopic noxious conditioning stimulation (HNCS).

	Baseline	During HNCS	5 min after	15 min after
Electrical pain threshold				
Finger (0–60 au), all subjects	8.4 (2.8)	12.6 (5.1)***	10.1 (3.4)**	9.6 (3.6)*
Males	9.4 (2.8)	14.4 (5.4)	11.2 (3.7)	11.1 (3.7)
females	7.5 (2.5)	10.8 (4.2)	9.0 (2.6)	8.1 (2.9)
Incisor (V), all subjects	26.0 (8.6)	25.9 (7.3)	26.2 (9.7)	24.5 (9.5)
Males	29.8 (8.9)	27.1 (7.8)	29.3 (11.0)	27.7 (10.1)
females	22.3 (6.5)#	24.7 (6.8)	22.9 (7.2)	21.3 (7.9)
Pressure pain threshold				
Finger (kPa), all subjetcs	475 (156)	570 (221)***	498 (193)	467 (189)
Males	555 (135)	654 (236)	591 (209)	552 (211)
females	395 (136)#	486 (174)	399 (112)	383 (119)
Masseter muscle (kPa), all subjects	200 (74)	291 (117)***	220 (76)***	211 (68)***
Males	216 (72)	286 (87)	235 (78)	222 (67)
females	184 (75)	296 (144)	204 (74)	200 (70)

Au = arbitrary units. # = Significant difference between genders ($P<0.05$). * = Significant difference compared to baseline (*** = $P<0.001$; ** = $P<0.01$; * = $P<0.05$).

no differences in pain thresholds between subjects tested on the right or left side.

Changes of pain thresholds by HNCS

The averaged pain thresholds before, during, and after HNCS are given in Table I. All pain thresholds except for EPT in the tooth were increased during the HNCS and thereafter declined to baseline during the 15 min after the HNCS.

In the first ANOVA, the effect of gender and time on pain thresholds was tested for each stimulus and region separately. No significant differences were found between genders for any stimuli or region and there was no interaction between time and gender. However, the EPT in the finger differed significantly between time-points ($F=17.681$; $p<0.001$; Figure 1A). The post-hoc test showed that it was significantly increased during and 5 and 15 min after the HNCS compared to baseline. The EPT in the tooth, on the other hand, did not differ significantly between time-points (Figure 1B). The PPT in the finger differed significantly between time-points ($F=13.785$; $p<0.001$; Figure 1C) and the post-hoc test showed that it was higher during the HNCS than at baseline. Also the PPT in the masseter muscle differed between time-points ($F=21.419$; $p<0.001$; Figure 1D), with the post-hoc test showing that it was significantly higher during the HNCS and 5 and 15 min after HNCS than at baseline.

In the second ANOVA, the effect of region and time was tested for each stimulus separately (Figure 2). The changes in EPT differed significantly between regions ($F=18.191$; $p<0.001$) and there was an interaction between region and time ($F=11.526$; $p<0.001$). The post-hoc test showed that the changes in the finger were greater than those in the tooth during and 5 and 15 min after the HNCS. Also the changes in PPT differed between regions ($F=11.995$; $p=0.002$) and there was an interaction between region and time ($F=5.855$; $p=0.001$). The post-hoc test revealed that PPT changes in the masseter muscle were greater than those in the finger during and 15 min after the HNCS.

In the last ANOVA, the effect of stimulus and time was tested for each region separately (Figure 2). In both the finger and orofacial regions the changes in pain thresholds differed significantly between stimuli (finger: $F=21.427$; $p<0.001$; orofacial: $F=17.012$; $p<0.001$). There were also interactions between stimuli type and time in both regions ($F=23.967$ and $F=14.366$; $p<0.001$ for both). In the finger, the changes in pain thresholds were greater for electrical stimuli than for mechanical stimuli during and 5 and 15 min after the HNCS. In the orofacial region, too, the changes in pain thresholds were greater for mechanical stimuli than for electrical stimuli during the HNCS.

There were no significant differences between subjects tested on the right versus left side nor dependent on the sequence of testing, i.e. whether the finger or orofacial region was tested first.

Pain induced by HNCS

The HNCS quickly induced pain of high intensity in all subjects, i.e. pain varying between 5 and 10 on the NRS, with a median (IQR) intensity of 8 (1.5). Pain unpleasantness also varied between 5 and 10, with a median (IQR) of 8.5 (2). Tolerance, i.e. the time that the subjects could tolerate their hand immersed in the cold water, varied between 64 and 300 s, with a mean (SD) of 196 (63) s. Two of the subjects endured the HNCS for the maximum time. There were no differences in the tolerance time with respect to gender. Pain intensity and pain unpleasantness did not differ between genders (Figure 3).

The tolerance time was positively correlated to the changes of PPT in the finger 15 min after HNCS

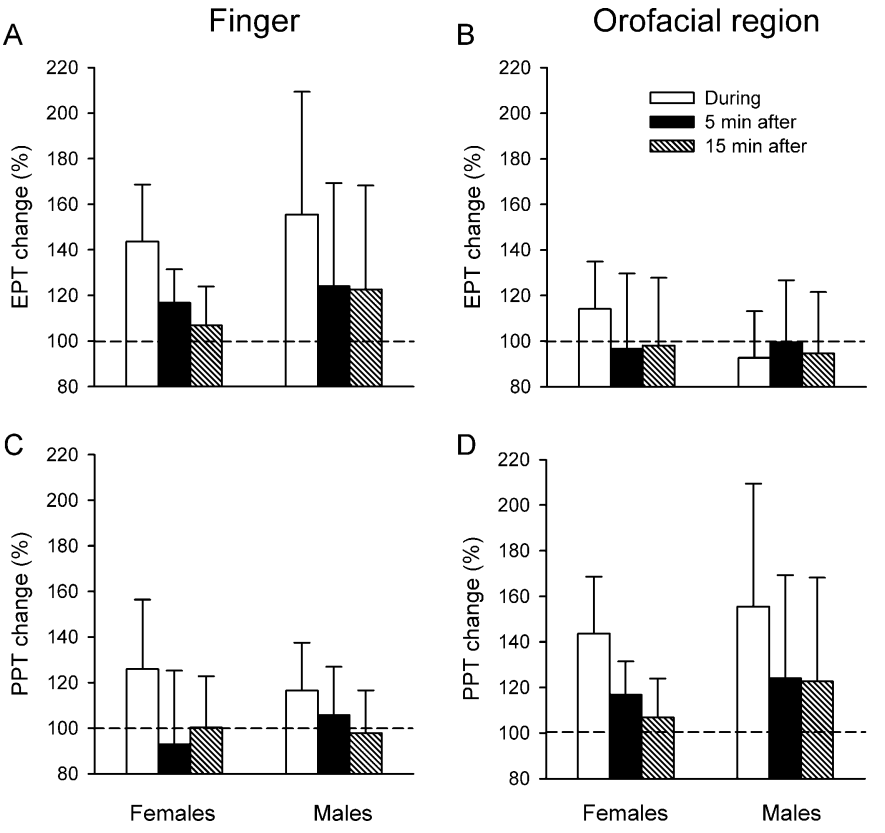

Figure 1. The mean (SD) relative changes to baseline (100%) of electrical (EPT) and pressure pain thresholds (PPT) in the finger and orofacial region during and 5 and 15 min after heterotopic noxious conditioning stimulation (HNCS) in 15 healthy female subjects and 15 age-matched male subjects. (A) EPT in the finger, (B) EPT in the orofacial region (incisor), (C) PPT in the finger, and (D) PPT in the orofacial region (masseter muscle). EPT and PPT in the finger and PPT in the orofacial region increased significantly during HNCS for the entire group compared to baseline ($p < 0.001$). EPT in the finger was also significantly increased compared to baseline 5 and 15 min after HNCS ($p = 0.008$ and $p = 0.038$, respectively), as was PPT in the masseter muscle ($p < 0.001$ for both time-points). There were no differences between genders in the response to HNCS.

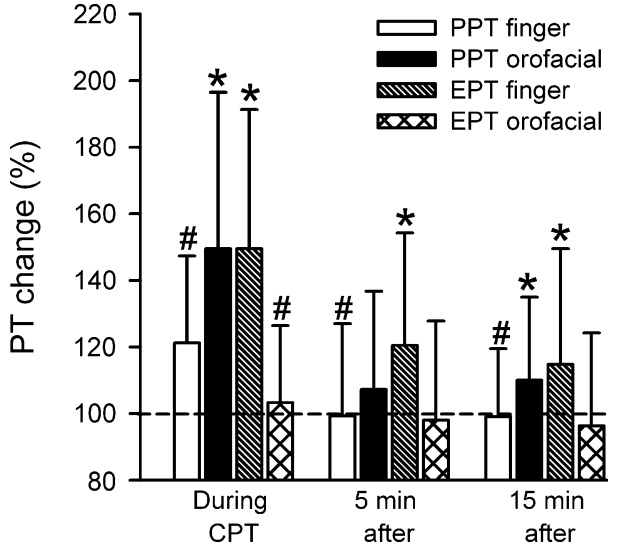

Figure 2. The mean (SD) relative changes to baseline (100%) of electrical (EPT) and pressure pain thresholds (PPT) in the finger and orofacial region during and 5 and 15 min after heterotopic noxious conditioning stimulation in 30 healthy subjects. *Significant difference between regions; #significant difference between stimulus type in the same region (Holm-Sidak method; $p < 0.05$).

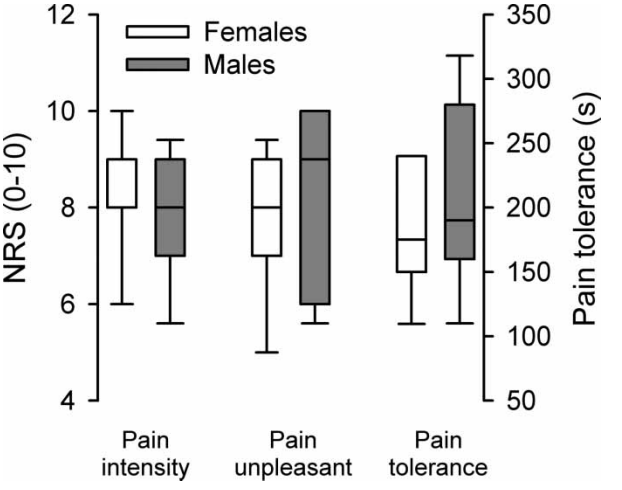

Figure 3. Median (IQR) pain intensity and pain unpleasantness assessed with a numeric rating scale (NRS, range 0–10) as well as tolerance time (s) to heterotopic noxious conditioning stimulation in 15 healthy female subjects and 15 age-matched male subjects. There were no differences between genders.

($r = 0.474$, $p < 0.05$), but there were no other correlations between pain intensity, unpleasantness or tolerance time and changes of pain thresholds during or after the HNCS.

Discussion

This study has shown that pain thresholds to electrical and pressure stimulation increased during HNCS. However, there was a greater increase of the EPT in the finger than in the orofacial region, and greater increase of PPT in the orofacial region than in the finger. In addition, electrical stimulation in the finger induced a greater change of pain thresholds than pressure, while the opposite was the case in the orofacial region. No gender differences in pain modulation were observed.

It is widely accepted that non-noxious and noxious stimulation can suppress pain. This could be due to the inhibition of more slowly conducting nociceptive afferents by activation of large diameter sensory afferents or to activation of endogenous pain inhibitory systems. Similar to a great number of other studies [6–11,26–28], the pain thresholds in the present study were generally increased during and after the HNCS, except for the EPT in the tooth. It is possible that this was due to DNIC effects, i.e. the concept that one noxious stimulus inhibits the perception of a painful sensation caused by another noxious stimulus. Another possibility is the release of endogenous opioids; it has been shown by others that naloxone increases pain intensity compared to placebo during HNCS in healthy subjects [29].

A long pain tolerance time was associated with a large change of PPT in the finger. This might indicate that subjects who could endure the HNCS the longest received more pronounced pain modulation. However, this was not seen for electrical stimuli nor for any stimuli in the orofacial region and there were no correlations between pain intensity or unpleasantness and changes of pain thresholds induced by the HNCS. In addition, the pain thresholds assessed during the HNCS would not be affected by this, since these were always measured 30 s after the hand had been immersed in the water bath.

The most interesting findings from this study are probably that there are tissue and site differences as well as stimulus-specific differences in pain modulation. This is in accordance with earlier studies, where it has been shown that nociception from myofascial tissue contributes to the pressure pain threshold or is dependent on which side of the body, the dominant or non-dominant side, the algometer is used on [30,31]. A previous study concluded that there are no segmental differences in pain perception at the spinal level, but differences have been reported between changes of pain thresholds at homotopic and heterotopic sites after intramuscular injection of hypertonic saline [32]. Other studies have reported differential effects between the magnitude and area of hyperalgesia at heterotopic sites [33] and sitedependent decreases of pain intensity at different spinal heterotopic sites [34]. To our knowledge, no previous study has investigated differences between spinal and trigeminal territories with respect to pain modulation by HNCS and only a few studies have investigated HNCS effects in the orofacial region [13,14,28,35,36]. They have all reported suppression of orofacial pain, similar to findings in the masseter muscle in the present study. In contrast, we did not reach significant changes in pain threshold during or after the HNCS as has been reported before where heterotopic ischemic stimulation attenuated pain and stimulus evoked potentials induced by electrical tooth stimulation [28]. In addition, heterotopic cold pressor pain was reported to suppress capsaicin-induced gingival pain [14]. Albeit the type of conditioning stimuli is known to influence the magnitude of pain modulation [28], this is an unlikely explanation for the difference between studies, since both ischemic and cold pressor stimuli induce pain of high intensity that is mediated by the same type of afferents (Aδ and C-fibers). However, one explanation could be that different methods of pain assessment were used. In studies by Fujii et al. [28] and Baad-Hansen et al. [14], a painful test stimuli was used, in contrast to the present study where we measured the pain threshold defined as the least level of stimulation required producing the first perception of pain.

Our results indicate differences in pain modulation within and between the two regions depending on the type of test stimulus. We found a more efficient suppression of painful mechanical stimuli in the masseter muscle than in the finger and a more efficient suppression of electrical stimuli in the finger than in the tooth. Conversely, mechanical stimuli in the orofacial region were suppressed more efficiently than electrical stimuli and vice versa in the finger. These findings point to differences in pain modulation between trigeminal and spinal innervated areas. This might partly explain the various results concerning HNCS effects in studies using different types of test and conditioning stimuli. One explanation could be that it is a shorter conduction distance of the peripheral pathways from the orofacial region than from the finger. Furthermore, electrical stimulation can be a more perceptive and more powerful stimuli, since it bypasses the receptor and therefore gives a different sensation in a fingertip from in a tooth. On the other hand, the tooth consists of unyielding hard tissue (enamel and dentine) and is one of the most densely nerve innervated areas in the body. Therefore, the tooth might not be a representative orofacial area for this type of experiment.

There seems to be consensus that there are gender differences to experimental pain [15,16]. At baseline, the men in this study showed 17–40% higher pain threshold to electrical and pressure stimuli than the women did. In general, our results thus concur with previous findings of lower pain thresholds in women than in men [30,31,37,38], although other studies in the orofacial region have not found any gender differences. The differences between studies in pain thresholds could be related to various sample sizes [37].

Bragdon et al. [39] have shown a correlation between increased plasma levels of β-endorphin and higher ischemic pain tolerance in women but not in men. They suggested that there are gender differences in the modulation of pain; women rely on opioid mechanisms and men on autonomic mechanisms. In the present study, no gender differences in the changes of pain thresholds during HNCS were seen. It could be argued that the sample size was too small for such differences to be detected, although previous studies have shown gender differences in pain modulation with even fewer subjects [10,11,15]. In addition, there are conflicting results as to whether there are gender differences in endogenous pain modulation [8–14,39]. This might be attributed to many factors, such as biological, psychological, and behavioral factors as well as methodological differences between studies.

Finally, certain methodological issues need to be addressed. One concern that can be raised is that two different devices were used to assess electrical sensitivity in the finger and tooth. However, the magnitude of the changes in the PPT of the masseter muscle and finger differed during HNCS, although the same algometer was used. Furthermore, the relative changes from baseline at each site were used in the statistical analyses, i.e. the magnitude of the changes should be comparable. Furthermore, no control session was used in the present study and therefore we cannot be certain that it was the HNCS that caused the suppression of pain. On the other hand, several previous placebo-controlled studies have shown pain modulator effects by HNCS in contrast to non-noxious stimulation [9,13]. It is also possible that psychological effects could have influenced our results. However, HNCS effects are reported to be attention insensitive. In addition, the aim of this study was not to investigate the difference between placebo and HNCS induced pain suppression or the influence of psychological effects, but whether the gender, region, tissue, or stimulus type influenced pain modulation.

In conclusion, activation of the endogenous pain inhibitory control system increases the pain thresholds at heterotopic sites, but the magnitude of the effect differs between the orofacial region and finger, and is influenced by the tissue and type of test stimuli. The results may have implications for the design of future studies and for our understanding of the complexity of orofacial pain.

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