Painful Conditioning Stimuli of the Craniofacial Region Evokes Diffuse Noxious Inhibitory Controls in Men and Women

Kelun Wang, DDS, PhD
Associate Professor
Center for Sensory-Motor Interaction
Orofacial Pain Laboratory
Aalborg University
Department of Oral and Maxillofacial Surgery
Aalborg Hospital
Aalborg, Denmark

Peter Svensson, Dr Odont, PhD, DDS
Professor
Department of Clinical Oral Physiology
School of Dentistry
University of Aarhus
Aarhus C, Denmark

Barry J. Sessle, MDS, PhD, DSc(hc)
Professor and Canada Research Chair
Faculty of Dentistry
University of Toronto
Toronto, Ontario, Canada

Brian E. Cairns, PhD, RPh
Associate Professor and Canada Research Chair in Neuropharmacology
Faculty of Pharmaceutical Sciences
University of British Columbia
Vancouver, British Columbia, Canada

Lars Arendt-Nielsen, PhD, Dr Med Sci
Professor
Center for Sensory-Motor Interaction
Orofacial Pain Laboratory
Aalborg University
Aalborg, Denmark

Correspondence to:
Dr Kelun Wang
Associate Professor
Center for Sensory-Motor Interaction
Aalborg University
Fredrik Bajers Vej 7 D-3
DK-9220 Aalborg E, Denmark
Fax: + 45 9815 4008
Email: kelun@smi.auc.dk

Aims: *To compare the modulatory effects of tonic mechanical or thermal craniofacial painful conditioning stimuli on pain sensitivity in craniofacial and spinal test sites in healthy men and women.* ***Methods:*** *Mechanical and cold headbands were developed and tested on 12 healthy men and 12 age-matched women (mean ± SEM: 27 ± 1.5 years). The pressure applied by the mechanical headband around the skull above the eyebrows could be adjusted over time via feedback from a 0 to 10 electronic visual analog scale (VAS) to maintain the pain intensity at a given level for 10 minutes (3 to 7 on VAS). The cold headband consisted of a series of plastic bags filled with antifreeze water having a temperature of approx 3°C. During the 10 minutes of application, the subjects were asked to rate the pain intensity on a 10-cm VAS. Pressure pain thresholds (PPT) were recorded over the right and left masseter muscles (MAR, MAL), right splenius muscle (neck), right elbow (elbow), and right middle finger (finger) by a pressure algometer (1-cm^2 area probe). The PPTs at each of the five sites were determined at baseline and during the mechanical or cold-induced pain. The two sessions with mechanical or cold headbands were performed at an interval of 30 minutes.* ***Results:*** *Women had significantly lower absolute PPT values than men at most test sites (Unpaired t-test:* $P < .027$*). The mechanical headband caused pain in both men (peak pain mean ± SEM: 4.7 ± 0.4 cm) and women (4.9 ± 0.4 cm) (*$P = .455$*). A significant PPT elevation was found at MAR, MAL, neck, and finger in men (11% to 17%;* $P < .031$*) and at MAR, MAL, and neck in women (15% to 22%;* $P < .020$*) during the mechanical-induced pain. The cold headband caused pain in both men (4.0 ± 0.4 cm) and women (4.5 ± 0.4 cm) (*$P = .285$*). During the cold-induced pain, a significant PPT elevation was found at all test sites in men (*$P < .023$*) and at all sites (*$P < .021$*) except for the finger in women. The relative changes in PPT values were not significantly different between men and women at any test site (unpaired t-test:* $P > .446$*).* ***Conclusion:*** *This study has documented that mechanical and thermal painful tonic stimuli applied to the craniofacial region can evoke diffuse noxious inhibitory control (DNIC)-like effects in the craniofacial region as well as spinally innervated areas, but without sex differences.* J OROFAC PAIN 2010;24:255–261

Key words: DNIC, human experimental pain models, sensory physiology, trigeminal pain

Studies in animal and human experimental pain models have shown that diffuse noxious inhibitory controls (DNIC) may contribute to pain-modulatory effects.[1–9] Their clinical significance lies in documentation that dysfunction of these inhibitory mechanisms and/or of related facilitatory mechanisms may be important factors in the development and maintenance of several chronic pain states, many of which have a female predominance.[10–14] However, most of the DNIC research focus has been

on the spinal nociceptive system, rather than the craniofacial nociceptive system, and most studies have been performed in men. Studies comparing DNIC in men and women have so far shown conflicting results. Similar DNIC effects in both men and women have been reported in two previous studies using electric stimulation as the test stimulus[15] or intraoral capsaicin,[16] whereas sex-related differences have been reported in the other studies.[13,17] One reason for the conflicting data could be due to differences in the modality of the conditioning stimulus. To date, no human studies have focused on sex differences in DNIC evoked by different conditioning stimulus modalities applied to the craniofacial region.

The aim of this study was to compare the modulatory effects of tonic mechanical or thermal craniofacial painful conditioning stimuli on pain sensitivity in craniofacial and spinal test sites in healthy men and women.

Materials and Methods

Subjects

Twelve men and 12 healthy age-matched women (mean age ± SEM: 27 ± 1.5 years) participated in the experiment. The subjects had no signs or symptoms of temporomandibular disorders (TMD) in accordance with the Research Diagnostic Criteria for TMD[18] and were recruited among university students. The study protocol was approved by the local ethics committee in Denmark (VN: 2008036) and followed the guidelines set out by the Helsinki Declaration. Informed consent was obtained from all subjects before study inclusion.

Experimental Protocol

Tonic noxious mechanical (conditioning) stimulation was applied by a mechanical band, which could be tightened around the skull above the eyebrows and the pressure could be adjusted over time. The subjects were asked to rate the pain intensity continuously on a 0 to 10 electronic visual analog scale (VAS). Tonic noxious cold conditioning stimulation was applied by a series of plastic bags filled with antifreeze water having a temperature of approximately 3°C. After 10 minutes of application of the thermal headband, the subject was asked to rate the pain intensity on a 10-cm VAS. The pressure pain threshold (PPT) was recorded for right and left masseter muscles (MAR, MAL), right splenius muscle (neck), right elbow (elbow), and right middle finger (finger) with a pressure algometer (Somedic). The PPTs (ie, the amount of pressure [kPa] at which the subjects first perceived pain) at each site were determined in triplicate at baseline (ie, before headband placement) and then in duplicate with approximately 1 minute between each repeated measurement. The headbands were removed immediately after the PPT recordings. The two sessions with mechanical or cold headbands were performed in a randomized sequence on the same day at an interval of at least 60 minutes.

Tonic Mechanical Painful Stimulation

Tonic mechanical stimulation was applied by a customized mechanical band, which could be tightened around the skull above the eyebrows and the pressure could be adjusted over time (Fig 1a). Three different sizes of the headband (16, 18, and 20 cm in diameter) were made for fitting the individual head. Firm compression around the skull has previously been reported to cause a slowly increasing dull, deep pain sensation mimicking headache.[19] The force applied to the head was measured at one site on the skull (Fig 1a) with a pressure algometer (Somedic) and adjusted using the VAS feedback from the subjects to maintain pain intensity at 3 to 7 on the VAS. The pain intensity was rated by the subjects during the experiment. The headband was kept in the position for 10 minutes. The pain disappeared almost instantaneously (within a few minutes) when the headband was removed.

Tonic Cold Painful Stimulation

The cold pain stimulator was developed (Fig 1b) as a headband comprising a series of plastic bags filled with antifreeze water having a temperature of approximately 3°C. The bag headband with a width of 4 cm lightly covered the forehead, temple region, and back of the head, and was kept in position for a total of 10 minutes. The application time of each bag was based on the pain intensity rating on the VAS feedback of the subjects, so as to maintain VAS pain intensity at 3 to 7. This required the bag headband to be replaced with a fresh one approximately every 3 minutes. The cold pain disappeared soon after the headbands were removed.

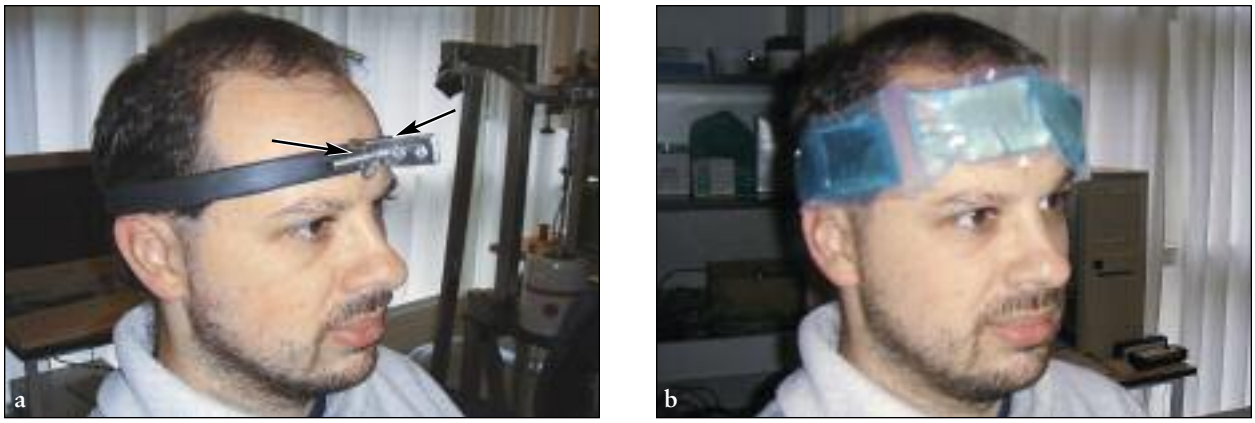

Fig 1 The mechanical *(a)* and cold *(b)* headbands used in the present study. *Arrows* indicate where the applied force was measured.

PPT

A pressure algometer (Somedic) was used to measure the PPTs. The PPT was defined as the amount of pressure (kPa) at which the subjects first perceived pain.[20] The algometer probe (1-cm^2 area) was applied perpendicularly to MAR, MAL, neck, elbow, and finger before and during the conditioning stimulation. The pressure was delivered with a constant application rate of 30 kPa/s.[20] The subject pushed a button to stop the pressure stimulation when the threshold was reached.

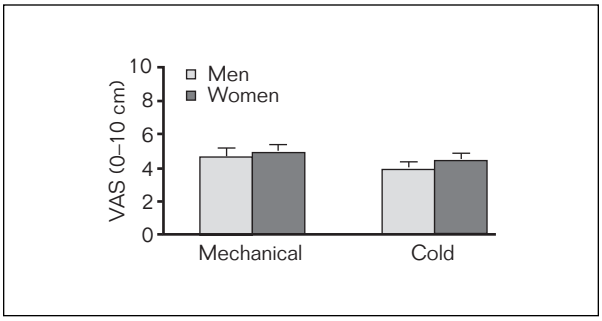

Fig 2 The mechanical and cold headband-evoked pain intensity rated on a VAS by the 24 volunteers (12 men and 12 women) (mean ± SEM).

Statistical Analyses

The sample size was calculated with a risk of type I and type II errors of 5% and 20%, respectively, and a conservative estimate of the intraindividual variation of 30% on the PPTs and a minimal relevant difference to detect as 25%. Thus, a total of 24 subjects were included.

Mean values ± SEM are presented in the text and figures. A three-way ANOVA was performed on the absolute PPT values to test the effect of time (baseline, during); locations, (MAR, MAL, neck, elbow, finger); and sex (men, women). Then, the PPT values were normalized to the baseline value and an additional two-way ANOVA was performed on the normalized data to test the possible time and sex effect on the relative changes of the PPT values. The relative changes at each point were also compared between sexes with unpaired *t*-tests. A paired *t*-test was performed to compare PPT values before (baseline) and during application of the conditioning stimulation for both groups of subjects to test whether the mechanical or cold stimulation altered the PPTs. Unpaired *t*-tests were also used to compare VAS pain evoked by the mechanical and cold conditioning stimuli between men and women. The significance level was set at $P < .05$.

Results

Headband-evoked Pain

The mechanical headband (Fig 1a) caused pain in all subjects. The force applied around the skull was 324 ± 44 kPa for men and 313 ± 38 kPa for women, with no significant differences between the two groups ($P > .264$). The peak pain intensity evoked by the mechanical headband was 4.7 ± 0.4 cm in men and 4.9 ± 0.4 cm in women ($P = .585$) (Fig 2).

The cold headband (Fig 1b) also caused pain in men (4.0 ± 0.4 cm) and women (4.5 ± 0.4 cm) ($P = .285$) (Fig 2). There were no differences in the VAS pain scores evoked by the mechanical or cold stimulation in men ($P = .777$) or in women ($P = .914$).

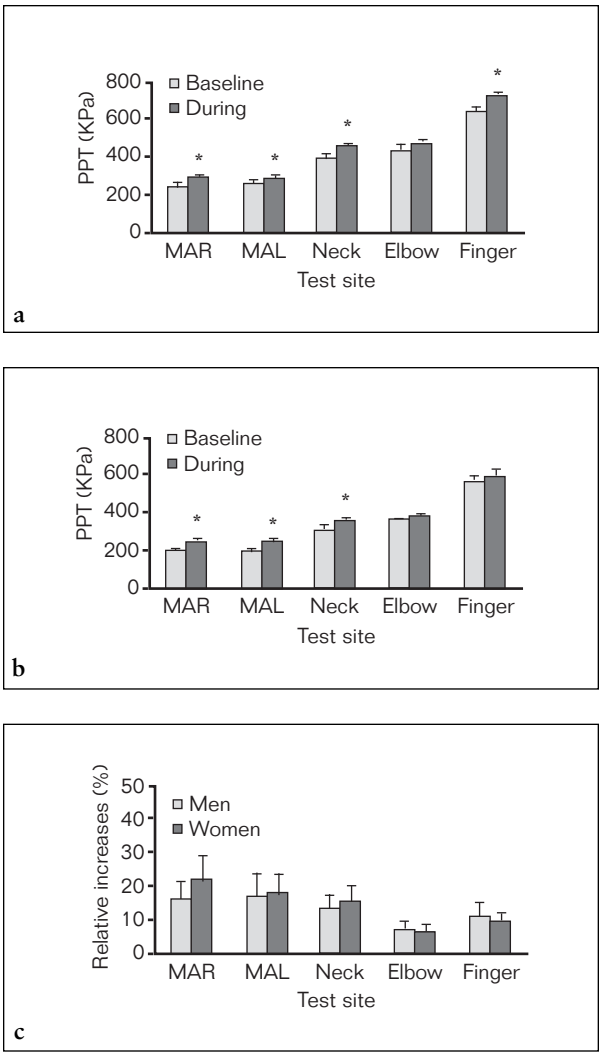

Fig 3 PPT values before (baseline) and during the application of the mechanical headband at MAR, MAL, neck, elbow, and finger (mean + SEM). *indicates significant increases compared to baseline for 12 men *(a)* and 12 women *(b)* ($P < .05$). There were no significant differences for the relative increases (%) of PPT during the mechanical headband application between men and women *(c)*.

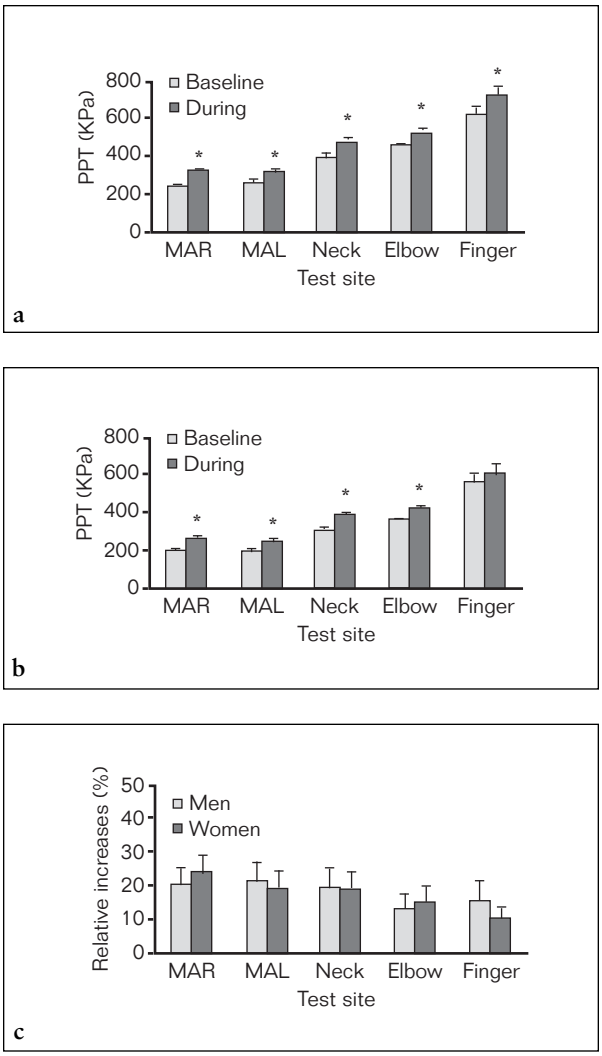

Fig 4 PPT values before (baseline) and during the application of the cold headband at MAR, MAL, neck, elbow, and finger (mean + SEM). *indicates significant increases compared to baseline for 12 men *(a)* and 12 women *(b)* ($P < .05$). There were no significant differences for the relative increases (%) of PPT during the cold headband application between men and women *(c)*.

PPT Findings

Three-way ANOVA showed significant main effects of locations, time, and sex ($P < .01$) on the absolute PPT values. At baseline, women had significantly lower PPT values than men at the MAR, MAL, and neck (unpaired *t*-test: $P < .027$). An additional two-way ANOVA on the normalized PPT values showed a significant time effect ($P < .028$), but no sex effect ($P > .464$).

Mechanical Stimulation. It was a consistent finding that PPTs during mechanical painful stimulation increased at all sites in men except at the elbow ($P < .031$) (Fig 3a) and in women except at the elbow and finger ($P < .020$) (Fig 3b). There were no significant sex-related differences in the relative increases at any test site (unpaired *t*-test: $P > .446$) (Fig 3c).

Cold Stimulation. For painful cold stimulation, there were significant increases in PPT values at all test sites in men ($P < .023$) (Fig 4a) and in women except at the finger ($P < .021$) (Fig 4b). There were no significant sex-related differences in relative increases at any test site (unpaired *t*-test: $P > .164$) (Fig 4c).

Discussion

The technique described in this paper allowed painful tonic mechanical and thermal conditioning stimuli to be applied to the craniofacial region. These conditioning stimuli induced DNIC-like responses, as reflected in significantly increased PPT values at all craniofacial as well as in most spinally innervated test sites in both men and women. No sex-related difference was detected in the magnitude of the DNIC-evoked increases in PPT values.

Methods and Study Limitations

To the best of the authors' knowledge, this is the first human study to have applied noxious conditioning stimuli to the craniofacial region in order to compare DNIC responses in both craniofacial and spinally innervated areas. There are, however, some limitations of the current methodology that may have confounded interpretation of the results. Three different sizes of the headband were used, and the subjects could select a suitable size according to the size of their head. The force applied to the head was measured at one site of the skull (see Fig 1a), but the distribution of the pressure to the skin/skull could have been different from subject to subject because of differences in head shapes. In addition, most women had more hair at the site of mechanical stimulation, which might have affected the applied force. Furthermore, only healthy young subjects were tested in the present study and; hence, possible age-related differences in the DNIC responses were not examined.[21] The limited number of subjects (12 in each group) recruited in the present study could have affected the detection of possible sex differences and could explain why some of the PPT values (eg, at elbow and finger) showed no significant elevation during DNIC stimulation. Larger study groups will be needed to clarify sex-related differences in DNIC responses, but the present study did not indicate major differences in the magnitude of the DNIC responses, although at the spinal test sites women had fewer consistent increases in PPT values than men. Finally, a "postpain" session was not included in the present design. Further studies will be needed to address the time course of the DNIC effects evoked from the craniofacial region.

DNIC Studies in Acute Craniofacial Pain Models

It has been shown from animal studies that stimulation of not only craniofacial areas but also remote parts of the body induces modulation of jaw and tongue inhibitory reflex responses in animals[22–24] and humans[25,26] to noxious craniofacial stimuli. DNIC effects are also powerfully expressed in both nociceptive-specific as well as wide dynamic range neurons in the trigeminal brainstem sensory nuclear complex, such as its subnucleus caudalis, and are also reflected in sensorimotor behavioral responses in which the caudalis participates.[27–30]

The heterotopic character of DNIC has been demonstrated in many studies of human volunteers.[1–9] Painful heterotopic conditioning stimuli (thermal, mechanical, electrical, or chemical) decrease pain perception induced by phasic noxious stimulation applied elsewhere in the body.[7,8,31] A recent human study has documented that noxious cold stimulation of the hand induces DNIC effects on capsaicin-evoked intraoral pain.[16] The late component of somatosensory evoked potentials induced by electrical painful tooth stimulation can also be inhibited by heterotopic ischemic stimulation of the upper arm.[32] DNIC evoked from the limb also produces inhibition of nociceptive trigeminal-mediated reflex responses.[33–35] So far, it remains to be determined if craniofacial stimulation-evoked DNIC in healthy humans has a similar nature and potency as DNIC evoked from spinally innervated areas.

Sex Differences in DNIC Responses

It is still unclear if there are sex differences in DNIC as the literature shows conflicting results. In accordance with the present findings, similar magnitudes of DNIC effects in both men and women have been reported in two studies using electrical stimulation[15] or intraoral capsaicin[16] as the test stimulus. However, sex differences in DNIC have been reported in studies using thermal stimulation of the hand[13] or noxious stimulation of the trapezius muscle.[17,36] Both studies reported longer-lasting hypoalgesia in men than in women. A recent study comparing DNIC evoked by hypertonic saline-induced (6%) muscle pain (tibialis anterior) or cold pressor pain between men and women showed that cold pressor pain increased PPT in both men and women, with greater increases in men; hypertonic saline-evoked muscle pain significantly increased PPT in men but not in women.[37] These data raise the possibility that muscle pain-induced DNIC may be sex-dependent, whereas DNIC induced by

noxious stimulation of the forearm or oral mucosa may not.[15,16] It is also conceivable that the modality (thermal, mechanical, or chemical) and/or test site (skin, muscle, or viscera) of the test stimulation may be an important factor in determining whether effects of DNIC will be different in men and women.

Another consideration is the potential relationship between DNIC effects and psychological characteristics. It has been shown that depression and somatization are correlated with sensitivity to experimental painful stimuli[38] and clinical TMD pain.[39] Furthermore, catastrophizing scores are correlated with pain sensitivity and often in a sex-dependent manner.[40–42] However, so far, there have been no systematic attempts to correlate DNIC effects with such psychological measures.

Establishing new human models that explore the inhibitory processes of tonic and chronic craniofacial musculoskeletal pain is of utmost importance for improving our understanding of pain control. Human experimental pain models in healthy volunteers act as a bridge between animal and clinical studies, since the effect of specific test and conditioning modalities can be studied under standardized and controlled conditions. The headband model developed in the present study is effective in evoking craniofacial DNIC-like effects and could be a valuable tool for the study of the unique properties of DNIC induced by pain in the craniofacial region.

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References

1. Talbot JD, Duncan GH, Bushnell MC, Boyer M. Diffuse noxious inhibitory controls (DNICs): Psychophysical evidence in man for intersegmental suppression of noxious heat perception by cold pressor pain. Pain 1987;30: 221–232.
2. De Broucker T, Cesaro P, Willer JC, Le Bars D. Diffuse noxious inhibitory controls in man. Involvement of the spinoreticular tract. Brain 1990;113:1223–1234.
3. Arendt-Nielsen L, Gotliebsen K. Segmental inhibition of laser-evoked brain potentials by ipsi- and contralaterally applied cold pressor pain. Eur J Appl Physiol Occup Physiol 1992;64:56–61.
4. Graven-Nielsen T, Babenko V, Svensson P, Arendt-Nielsen L. Experimentally induced muscle pain induces hypoalgesia in heterotopic deep tissues, but not in homotopic deep tissues. Brain Res 1998;787:203–210.
5. Witting N, Svensson P, Arendt-Nielsen L, Jensen TS. Differential effect of painful heterotopic stimulation on capsaicin induced pain and allodynia. Brain Res 1998; 801:206–210.
6. Svensson P, Hashikawa CH, Casey KL. Site and modality specific modulation of experimental muscle pain in humans. Brain Res 1999;851:32–38.
7. Reinert A, Treede RD, Bromm B. The pain inhibiting pain effect: An electrophysiological study in humans. Brain Res 2000;862:103–110.
8. Bouhassira D, Danziger N, Atta N, Guirimand F. Comparison of the pain suppressive effects of clinical and experimental painful conditioning stimuli. Brain 2003; 126:1068–1078.
9. Serrao M, Rossi P, Sandrini G, et al. Effects of diffuse noxious inhibitory controls on temporal summation of the RIII reflex in humans. Pain 2004;112: 353–360.
10. Carlsson GE, LeResche L. Epidemiology of temporomandibular disorders. In: Sessle BJ, Bryant PS, Dionne RA (eds). Temporomandibular Disorders and Related Pain Conditions. Progress in Pain Research and Management. Seattle: IASP, 1995:211–226.
11. Dao TT, LeResche L. Gender differences in pain. J Orofac Pain 2000;14:169–184.
12. Riley JL III, Gilbert GH. Orofacial pain symptoms: An interaction between age and sex. Pain 2001;90:245–256.
13. Staud R, Robinson ME, Vierck CJ Jr, Price DD. Diffuse noxious inhibitory controls (DNIC) attenuate temporal summation of second pain in normal males but not in normal females or fibromyalgia patients. Pain 2003;101: 167–174.
14. Sarlani E, Grace EG, Reynolds MA, Greenspan JD. Evidence for up-regulated central nociceptive processing in patients with masticatory myofascial pain. J Orofac Pain 2004;18:41–55.
15. France CR, Suchowiecki S. A comparison of noxious inhibitory controls in men and women. Pain 1999;81: 77–84.
16. Baad-Hansen L, Poulsen HF, Jensen HM, Svensson P. Lack of sex differences in modulation of experimental intraoral pain by diffuse noxious inhibitory controls (DNIC). Pain 2005;116:359–365.
17. Ge HY, Madeleine P, Arendt-Nielsen L. Sex differences in temporal characteristics of descending inhibitory control: An evaluation using repeated bilateral experimental induction of muscle pain. Pain 2004;110:72–78.
18. Dworkin SF, LeResche L. Research diagnostic criteria for temporomandibular disorders: Review, criteria, examinations and specifications, critique. J Craniomandib Disord 1992;6:301–355.
19. Wolff HG. Headache and Other Head Pain. New York: Oxford University, 1963.
20. Svensson P, Arendt-Nielsen L, Nielsen H, Larsen JK. Effect of chronic and experimental jaw muscle pain on pain-pressure thresholds and stimulus-response curves. J Orofac Pain 1995;9:347–356.
21. Larivière M, Goffaux P, Marchand S, Julien N. Changes in pain perception and descending inhibitory controls start at middle age in healthy adults. Clin J Pain 2007;23: 506–510.
22. Lowe AA, Gurza S, Sessle BJ. Excitatory and inhibitory influences on tongue muscle activity in cat and monkey. Brain Res 1976;113:417–422.

23. Gurza S, Lowe AA, Sessle BJ. Influences on masseter activity of stimuli applied to various sites in cats and macaque monkeys. Arch Oral Biol 1976;21:705-707.
24. Sessle BJ, Hu JW, Dubner R, Lucier GE. Functional properties of neurons in cat trigeminal subnucleus caudalis (medullary dorsal horn). II. Modulation of responses to noxious and nonnoxious stimuli by periaqueductal gray, nucleus raphe magnus, cerebral cortex, and afferent influences, and effect of naloxone. J Neurophysiol 1981;45: 193–207.
25. Dubner R, Sessle BJ, Storey TA. The Neural Basis of Oral and Facial Function. New York: Plenum Press, 1978.
26. Cadden SW. Modulation of human jaw reflexes: Heterotopic stimuli and stress. Arch Oral Biol 2007;52: 370–373.
27. Villanueva L, Le Bars D. The activation of bulbo-spinal controls by peripheral nociceptive inputs: Diffuse noxious inhibitory controls. Biol Res 1995;28:113–125.
28. Hu JW. Response properties of nociceptive and non-nociceptive neurons in the rat's trigeminal subnucleus caudalis (medullary dorsal horn) related to cutaneous and deep craniofacial afferent stimulation and modulation by diffuse noxious inhibitory controls. Pain 1990; 41:331–345.
29. Le Bars D. The whole body receptive field of dorsal horn multireceptive neurons. Brain Res Rev 2002;40:29–44.
30. Millan MJ. Descending control of pain. Prog Neurobiol 2002;66:355–474.
31. Bouhassira D, Bing Z, Le Bars D. Studies of brain structures involved in diffuse noxious inhibitory controls in the rat: The rostral ventromedial medulla. J Physiol 1993;463: 667–687.
32. Fujii K, Motohashi K, Umino M. Heterotopic ischemic pain attenuates somatosensory evoked potentials induced by electrical tooth stimulation: Diffuse noxious inhibitory controls in the trigeminal nerve territory. Eur J Pain 2006; 10:495–504.
33. Ellrich J, Treede RD. Characterization of blink reflex interneurons by activation of diffuse noxious inhibitory controls in man. Brain Res 1998;803:161–168.
34. Serrao M, Rossi P, Parisi L, et al. Trigemino-cervical-spinal reflexes in humans. Clin Neurophysiol 2003;114: 1697–1703.
35. Giffin NJ, Katsarava Z, Pfundstein A, Ellrich J, Kaube H. The effect of multiple stimuli on the modulation of the "nociceptive" blink reflex. Pain 2004;108:124–128.
36. Ge HY, Madeleine P, Arendt-Nielsen L. Gender differences in pain modulation evoked by repeated injections of glutamate into the human trapezius muscle. Pain 2005; 113:134–140.
37. Arendt-Nielsen L, Sluka KA, Nie HL. Experimental muscle pain impairs descending inhibition. Pain 2008;140: 465–471.
38. Sherman JJ, LeResche L, Huggins KH, Mancl LA, Sage JC, Dworkin SF. The relationship of somatization and depression to experimental pain response in women with temporomandibular disorders. Psychosom Med 2004;66: 852–860.
39. Yap AU, Chua EK, Tan KB, Chan YH. Relationships between depression/somatization and self-reports of pain and disability. J Orofac Pain 2004;18:220–225.
40. Keogh E, Eccleston C. Sex differences in adolescent chronic pain and pain-related coping. Pain 2006;123: 275–284.
41. Wijnhoven HA, de Vet HC, Picavet HS. Prevalence of musculoskeletal disorders is systematically higher in women than in men. Clin J Pain 2006;22:717–724.
42. Castrillon EE, Cairns BE, Ernberg M, et al. Effect of a peripheral NMDA receptor antagonist on glutamate-evoked masseter muscle pain and mechanical sensitization in women. J Orofac Pain 2007;21:216–224.